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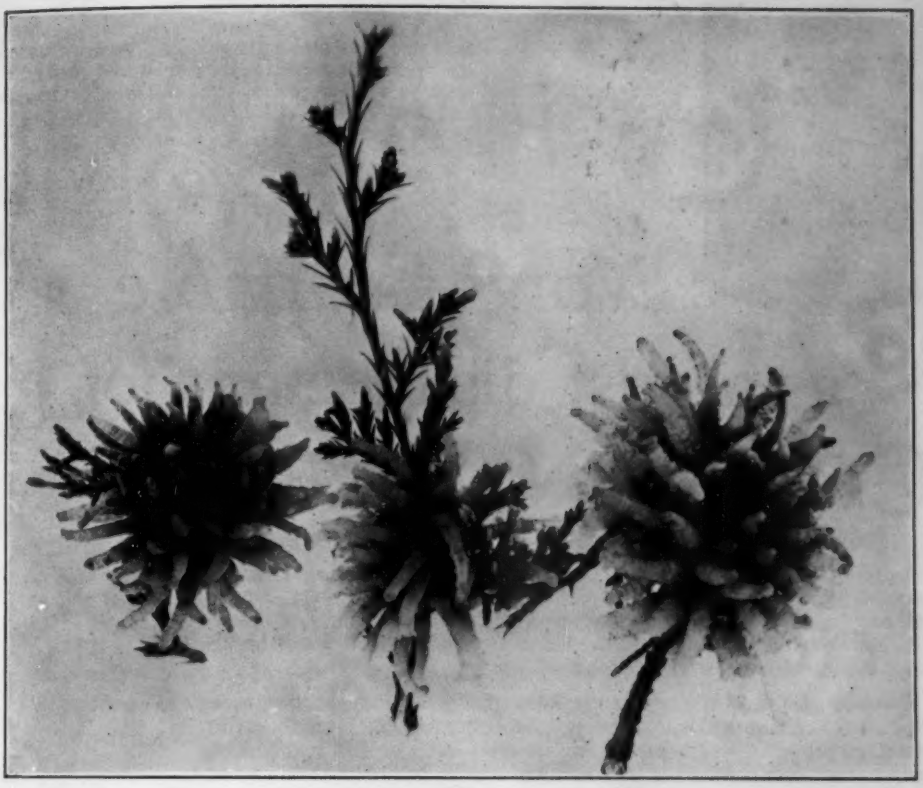

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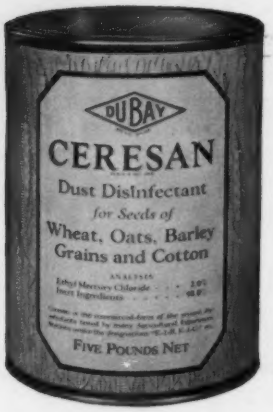

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DISEASE AND CLIMATE AS PERTAINING TO THE FLORIDA AND MAINE POTATO SECTIONS

L. O. GRATZ.

Approximately 26,000 acres of Irish potatoes are planted in Florida annually.[1] Over 19,000 acres of this total are planted in the Hastings area, which consists of a part of St. Johns, Putnam, Flagler, and Clay counties. Usually about 120,000 eleven-peck bags of State certified seed potatoes, valued at a half million dollars, are imported every year into Florida from Aroostook County, Maine. This amount of seed plants almost the entire acreage in the potato section proper.

The majority of the disease troubles of white potatoes are seed-borne. It is evident, other things being equal, that the Florida fields should exhibit symptoms of the same diseases as those observed in the Maine fields which produce the seed stock for this southern planting.

Careful field observations for 5 years, both in Florida and Maine, beginning in 1924 and ending in 1928, have demonstrated that some of the same troubles are found in both sections. Among the common diseases found in both States are late blight (*Phytophthora infestans* (Mont.) de Bary), early blight (*Alternaria solani* (E. & M.) J. & G.), rhizoctonosis (*Corticium vagum* B. & C.), scab (*Actinomyces scabies* (Thax.) Güssow), blackleg (*Bacillus atrosepticus* van Hall), and the various virus diseases (causes not determined). Some of these diseases do not produce such severe injury in Florida as in Maine. In the Hastings area, for example, scab and rhizoctonosis are not a commercial problem. Verticillium wilt (*Verticillium albo-atrum* Reinke and Berth.) is very prevalent during some seasons in Maine but has not been observed in Florida, and bacterial wilt (*Bacterium solanacearum* EFS.) is unknown in Maine but, at times, is very destructive in the southern potato sections.

These differences and the fact that, from the standpoint of the southern grower, the strategic point for the control of some of these diseases is in the seed-stock fields of the North have led to extensive observations in both sections, as reported herein.

[1] Average for 5 years, 1924–1928, as tabulated in U. S. Dept. of Agriculture, Crops and Markets, April, 1929, p. 123.

REVIEW OF LITERATURE

The relationship of environmental factors to the occurrence and severity of plant diseases has been recognized and studied for many years both by laymen and technical workers. Much experimental evidence to establish the exact facts regarding these relationships for plant pathogenes of all kinds has been accumulated both in this and other countries. Much work of this nature has been done in the laboratories of the Department of Plant Pathology at the Wisconsin Agricultural Experiment Station. An extensive summary, giving the results of experiments extending over a decade, with a complete citation of literature, is given by Jones, Johnson, and Dickson in their research bulletin No. 71 (9). Therefore, such a detailed review is unnecessary here.

Many field observations, confirmed by careful experimentation, have revealed that frequently small differences in mean temperature cause either slight attacks or severe epiphytotics, as the case may be. Such observations have likewise demonstrated that the geographic limitation of certain disease organisms may be due mostly to temperature differences. This is well illustrated in the above researches, as typified by cabbage yellows, flax wilt, rhizoctonosis of potatoes, and other diseases.

OBSERVATIONS AND INVESTIGATIONS

Climatological Data. Knowing that differences of environment cause variations in the severity of disease and observing that these differences in severity exist when Maine and Florida conditions are studied comparatively, the first step necessary to obtain more definite information concerning the relation of environment and potato diseases was to investigate climatic conditions during the respective growing seasons. Detailed weather records of these two sections were not readily available from previous publications. However, original data of the local weather observers were accessible in the Hastings belt, at Federal Point, Florida,[2] and at Aroostook Farm, Presque Isle, Maine. These figures were obtained and summarized. The records of the Florida observer date back 35 years, while those of Aroostook County cover a period of only 15 years. In order to make such climatological data for these two areas more readily available and of value for other investigations they are given here in complete detail (Tables 1–5; Figs. 1–3). From these original observations daily and monthly averages for the respective growing seasons were prepared for a period of 10 consecutive years both for Florida and Maine (Tables 1–3). In order that a clearer comparison may be made of the temperatures during the corresponding periods of the growing season the secular trends of the daily means were determined for both

[2] In the potato area 4 miles northwest of Hastings village.

TABLE 1.—*Daily average temperatures during the ten-year periods 1917–1926 and 1916–1926*[2] *for the potato-growing seasons at Federal Point, Florida,*[1] *and Aroostook Farm, Presque Isle, Maine, respectively*

FLORIDA				MAINE				FLORIDA				MAINE			
Date	Max.	Min.	Mean	Date	Max.	Min.	Mean	Date	Max.	Min.	Mean	Date	Max.	Min.	Mean
Jan. 15	67.0	45.4	56.2	May 15	60.4	35.6	48.0	Mar. 20	76.3	56.8	66.6	July 18	79.1	54.1	66.6
16	68.2	46.5	57.4	16	59.2	35.7	47.5	21	78.1	55.7	66.9	19	80.4	58.6	69.5
17	67.6	53.1	60.4	17	65.9	38.8	52.4	22	77.0	53.5	65.3	20	80.9	56.8	68.9
18	66.6	49.6	58.1	18	66.6	40.3	53.5	23	78.1	54.2	66.2	21	79.1	56.8	68.0
19	70.5	48.3	59.4	19	65.6	39.8	52.7	24	76.2	54.9	65.6	22	80.2	53.8	67.0
20	74.3	47.6	61.0	20	66.6	39.8	53.2	25	79.3	55.3	67.3	23	76.5	55.9	66.2
21	73.4	48.8	61.1	21	64.6	41.4	53.0	26	80.6	55.2	67.9	24	77.2	54.3	65.8
22	73.1	52.0	62.6	22	62.8	40.8	51.8	27	80.5	58.1	69.3	25	76.5	54.1	65.3
23	69.9	51.4	60.7	23	60.0	41.5	50.8	28	78.2	56.1	67.2	26	75.9	52.5	64.2
24	67.8	50.6	59.2	24	63.3	34.6	49.0	29	76.7	56.1	66.4	27	78.3	51.6	65.0
25	68.1	51.7	59.9	25	64.4	39.3	51.9	30	77.4	57.6	67.5	28	74.6	52.5	63.6
26	65.9	46.5	56.2	26	62.0	40.4	51.2	31	79.7	54.3	67.0	29	75.7	51.4	63.6
27	67.3	47.1	57.2	27	63.3	38.3	50.8	Apr. 1	77.1	55.9	66.5	30	75.9	53.7	64.8
28	69.3	51.0	60.2	28	68.3	39.1	53.7	2	76.8	52.5	64.7	31	77.6	56.2	66.9
29	69.1	50.1	59.6	29	69.3	41.5	55.4	3	79.1	53.2	66.2	Aug. 1	77.1	55.0	66.2
30	69.6	50.9	60.3	30	64.5	40.9	52.7	4	80.0	57.9	69.0	2	78.2	51.9	65.1
31	69.3	52.2	60.8	31	68.7	41.5	55.1	5	78.6	58.8	68.7	3	77.3	52.8	65.1
Feb. 1	70.0	49.6	59.8	June 1	71.9	43.3	57.6	6	77.4	57.0	67.2	4	77.1	49.6	63.4
2	67.3	49.8	58.6	2	68.7	41.6	55.2	7	78.9	56.0	67.5	5	77.7	49.7	63.7
3	65.9	46.4	56.2	3	71.8	46.1	59.0	8	81.4	56.0	68.7	6	78.8	52.2	65.5
4	67.1	48.6	57.9	4	74.8	50.6	62.7	9	79.5	56.8	68.2	7	73.2	57.8	65.5
5	67.2	49.7	58.5	5	74.9	48.9	61.9	10	78.5	58.3	68.4	8	74.3	56.9	65.6
6	67.4	46.0	56.7	6	73.6	47.4	60.5	11	77.8	54.9	66.4	9	75.6	52.7	60.0
7	69.0	44.1	56.6	7	73.1	45.0	59.1	12	79.5	55.7	67.6	10	76.5	53.0	60.4
8	69.0	45.3	57.2	8	71.2	49.4	60.3	13	81.1	57.5	69.3	11	77.5	54.1	65.8
9	70.7	46.9	58.8	9	70.0	45.9	58.0	14	79.8	56.2	68.0	12	75.6	51.5	63.6
10	71.0	44.9	58.0	10	70.2	46.5	58.4	15	81.0	58.0	69.5	13	75.3	54.5	64.9
11	71.2	44.9	58.1	11	68.0	46.8	57.4	16	81.0	59.8	70.4	14	75.3	53.6	64.5
12	71.0	46.8	58.9	12	68.8	47.8	58.3	17	83.0	59.5	71.3	15	73.7	51.9	62.8
13	72.7	48.6	60.7	13	72.7	45.8	59.3	18	82.7	58.8	70.8	16	78.4	53.9	66.2
14	74.7	50.3	62.5	14	74.2	47.6	60.9	19	82.3	56.8	69.6	17	76.5	53.0	64.7
15	75.1	50.4	62.8	15	71.6	47.9	59.8	20	83.5	55.6	69.6	18	73.5	49.8	61.7

[1] Florida observations taken at Federal Point, Florida, and Maine observations taken at Aroostook Farm, Presque Isle, Maine.
[2] Data for 1918 missing.

TABLE 1—(*Continued*)

FLORIDA				MAINE			
Date	Max.	Min.	Mean	Date	Max.	Min.	Mean
Feb. 16	70.7	45.7	58.2	June 16	68.2	45.7	57.0
17	69.6	47.4	58.5	17	67.3	46.5	56.9
18	70.6	51.5	61.1	18	67.3	46.6	57.0
19	71.4	49.8	60.6	19	71.3	49.8	60.6
20	72.4	50.3	61.4	20	74.2	47.8	61.0
21	73.5	50.8	62.2	21	73.3	50.2	61.8
22	74.3	49.2	61.8	22	71.6	49.0	60.3
23	74.5	50.9	67.7	23	74.9	47.6	61.3
24	75.5	52.0	63.8	24	75.8	49.1	62.5
25	75.0	51.9	63.5	25	75.7	49.1	62.4
26	74.0	52.2	63.1	26	77.3	50.1	63.7
27	72.5	51.8	62.2	27	76.1	50.0	63.1
28	73.7	53.4	63.6	28	74.3	53.4	63.9
Mar. 1	74.7	53.2	64.0	29	70.9	51.3	61.1
2	73.7	51.1	62.4	30	73.6	53.0	63.3
3	74.9	48.9	61.9	July 1	74.1	51.1	62.6
4	74.2	50.4	62.3	2	75.4	51.6	63.5
5	74.6	49.8	62.2	3	76.5	51.8	64.2
6	72.9	50.2	61.6	4	75.7	53.2	64.5
7	74.0	53.0	63.5	5	76.4	51.9	64.2
8	73.1	48.6	60.9	6	78.8	50.7	64.8
9	74.8	48.6	61.7	7	78.8	54.8	66.8
10	76.4	52.7	64.6	8	79.9	54.4	67.2
11	76.4	52.4	64.4	9	77.1	50.6	63.9
12	78.0	51.8	64.9	10	77.1	52.3	64.7
13	77.4	50.9	64.2	11	77.8	53.2	65.5
14	74.4	51.2	62.8	12	79.7	55.5	67.6
15	75.9	52.1	69.0	13	79.2	55.4	67.3
16	77.0	55.2	66.1	14	78.0	53.5	65.8
17	79.7	56.0	67.9	15	80.5	55.9	68.2
18	76.5	57.5	67.0	16	76.3	54.8	65.6
19	79.4	58.4	68.9	17	77.4	55.4	66.4

FLORIDA				MAINE			
Date	Max.	Min.	Mean	Date	Max.	Min.	Mean
Apr. 21	84.4	58.7	71.6	Aug. 19	75.0	52.1	63.6
22	84.1	59.3	71.7	20	74.7	51.0	62.9
23	84.1	60.0	72.1	21	70.2	49.4	59.8
24	83.7	58.7	71.2	22	71.9	47.5	59.7
25	83.4	57.1	70.3	23	73.5	52.2	62.9
26	85.6	61.6	73.6	24	73.2	54.2	63.7
27	82.2	60.5	71.4	25	74.9	54.0	64.5
28	86.1	56.4	71.3	26	70.8	52.5	61.7
29	85.4	59.2	72.3	27	72.1	49.7	60.9
30	83.5	59.4	71.5	28	72.8	51.3	62.1
May 1	82.8	59.4	71.1	29	73.0	52.8	62.9
2	83.7	60.7	72.2	30	73.5	53.2	63.4
3	80.6	60.1	70.4	31	71.4	50.9	61.2
4	84.2	58.9	71.6	Sept. 1	71.1	46.5	58.8
5	81.9	59.4	70.7	2	69.2	49.7	59.5
6	83.2	60.1	71.7	3	68.2	47.7	58.0
7	84.8	59.6	72.2	4	66.2	44.6	55.4
8	85.3	60.7	73.0	5	69.1	39.7	54.4
9	84.7	58.6	71.7	6	63.6	43.0	53.3
10	86.1	58.9	72.5	7	66.5	43.3	52.9
11	87.5	60.9	74.2	8	67.7	43.6	55.7
12	87.5	61.1	74.3	9	69.4	43.4	56.4
13	88.0	64.2	76.1	10	64.0	45.4	54.7
14	85.5	63.9	74.7	11	66.4	45.5	56.0
15	84.3	63.2	73.8	12	64.4	47.8	56.1
16	84.3	62.1	73.2	13	65.8	51.2	58.5
17	84.3	63.1	73.7	14	63.3	45.5	54.4
18	85.3	63.2	74.3	15	62.9	40.4	51.7
19	85.4	63.4	74.4	16	63.0	38.5	50.8
20	87.0	63.4	75.2	17	64.4	41.4	52.9
21	85.6	63.9	74.8	18	64.4	41.2	52.8
22	86.5	64.3	75.4	19	64.6	39.6	52.1

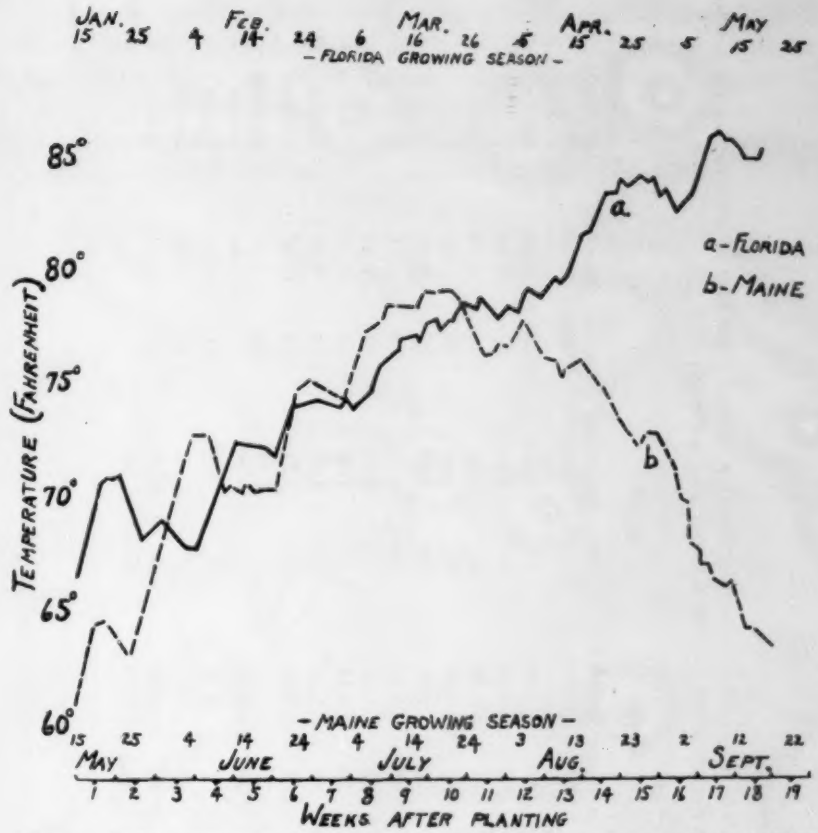

FIG. 1. Daily average maximal temperatures during the ten-year periods 1917–1926 and 1916–1926[1] for the potato-growing seasons at Federal Point, Florida, and Aroostook Farm, Presque Isle, Maine, respectively.

sets of data by plotting a daily moving average of the observations for every 7 successive days. The graphic representation of the normal temperatures thus obtained is shown in figures 1–3. Precipitation records are also given in tables 2–3.

The usual planting dates for most of the acreage in the Hastings and Aroostook County sections average about January 15 and May 15, respectively. It is noteworthy that the Florida temperatures for about the first 3 weeks after planting are higher than those in Maine during the corresponding period (Figs. 1–3). During the fourth week they are lower and the fifth and sixth week they may be slightly higher. From then on the Florida temperatures continue slightly lower until about the middle of the

[1] Data for 1918 missing.

TABLE 2.—*Late blight in relation to the average monthly temperatures and total monthly rainfall from 1914 to 1928 during the potato-growing season at Federal Point (in the Hastings area), Florida*

Year	January		February		March		April		May		Severity of late blight
	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	
1914	57	5.10	69	4.00	60	1.69	71	0.94	75	2.56	
1915	58	2.87	56	4.09	57	3.39	68	1.19	79	7.49	
1916	65	0.59	60	0.78	61	0.69	69	0.88	77	3.82	
1917	65	0.96	59	1.59	68	5.91	71	1.68	75	1.03	
1918	53	3.23	66	0.03	70	2.25	69	7.77	75	2.12	
1919	58	1.74	60	4.13	66	8.34	69	2.50	76	4.11	
1920	59	2.87	56	13.52	62	1.31	71	3.79	76	8.01	
1921	60	1.63	61	1.12	70	0.18	70	1.32	74	6.97	
1922	56	3.07	64	4.66	67	1.34	73	1.83	77	6.62	severe
1923	60	1.44	61	1.64	67	2.05	72	1.21	73	10.50	medium
1924	58	3.43	57	2.80	59	11.01	70	2.18	75	2.99	medium
1925	61	3.31	62	1.48	65	2.15	63	0.31	75	2.59	severe
1926	56	3.57	59	1.60	61	3.99	67	.....	74	0.85	severe
1927	58	0.25	66	3.81	65	1.57	72	1.04	77	1.66	severe
1928	55	0.92	61	1.64	66	2.39	68	8.38	73	4.22	none
Average	58.6	2.33	61.1	3.13	64.3	3.22	69.5	2.50	75.4	4.37	

season when the condition is reversed, and the cooler weather persists in the North and the higher temperatures in the South. With such a similarity in temperature from the third to the tenth week, little difference in the severity of certain diseases should be expected, if temperature is the primary governing factor. The actual conditions which have been observed in connection with the most common diseases are discussed below.

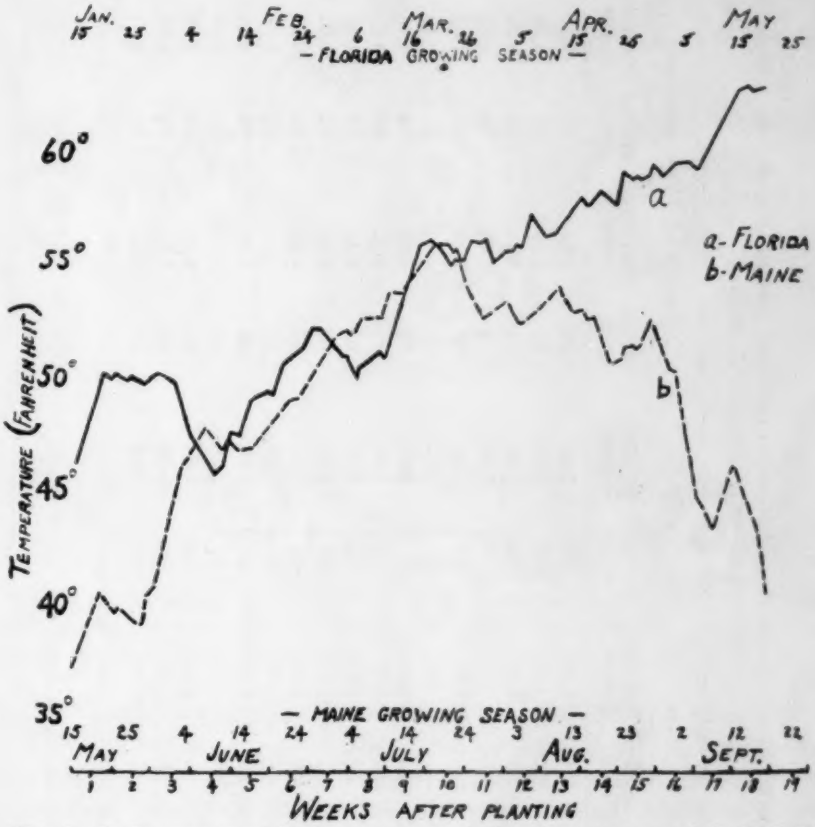
JAN. FEB. MAR. APR. MAY
15 25 4 14 24 6 16 26 5 15 25 5 15 25
— FLORIDA GROWING SEASON —
60°
55°
50°
45°
40°
35°
TEMPERATURE (FAHRENHEIT)
a
b
a-FLORIDA
b-MAINE
— MAINE GROWING SEASON —
15 25 4 14 24 4 14 24 3 13 23 2 12 22
MAY JUNE JULY AUG. SEPT.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
WEEKS AFTER PLANTING

FIG. 2. Daily average minimal temperatures during the ten-year periods 1917–1926 and 1916–1926[1] for the potato-growing seasons at Federal Point, Florida, and Aroostook Farm, Presque Isle, Maine, respectively.

FUNGOUS DISEASES

Late blight (*Phytophthora infestans* (*Mont.*) *de Bary*). The symptoms of this disease, under average conditions, are identical in both sections. By comparing those fields where proper control measures are not practiced one finds, occasionally, slightly greater destruction of foliage in Florida than in

[1] Data for 1918 missing.

TABLE 3.—*Late blight in relation to the average monthly temperatures and total monthly rainfall from 1913 to 1928 during the potato-growing season at Presque Isle, Maine*

Year	May		June		July		August		September		Severity of late blight[1]
	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	Average temp.	Rainfall inches	
1913	49	3.53	61	1.20	69	5.18	61	3.01	54	2.01	medium
1914	53	2.74	56	4.80	64	2.23	60	2.35	56	2.10	do
1915	51	4.05	61	1.95	64	3.40	62	3.50	56	3.25	severe
1916	51	3.45	60	2.17	68	3.68	72	1.70	59	4.05	do
1917	43	3.90	60	7.67	68	2.56	67	5.32	53	1.41	do
1918	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
1919	51	3.32	63	0.80	.....	3.80	61	1.75	53	4.56	10 per cent loss
1920	51	0.91	60	6.08	64	4.28	67	3.62	56	5.21	20 per cent do
1921	53	1.63	59	1.58	72	2.49	61	5.43	56	3.15	0.5 per cent do
1922	52	1.55	63	11.10	65	1.50	62	3.88	55	1.05	5 per cent do
1923	48	1.58	59	0.82	62	3.97	59	2.32	54	.....	0
1924	47	3.03	58	0.76	67	2.09	.....	.....	52	3.34	severe
1925	47	2.32	61	3.21	63	2.45	65	3.09	52	4.18	5 per cent loss
1926	46	1.86	58	1.84	67	2.10	61	3.71	52	3.30	7 per cent do
1927	47	2.08	53	2.32	66	2.94	61	5.25	55	1.36	25 per cent do
1928	50	2.59	56	3.06	66	4.62	65	3.30	52	4.80	severe
Average	49	2.57	59	3.29	66	3.15	63	3.45	54	3.13	

[1] 1913–1918 from Folsom (5).
1919–1926 from *Plant Disease Reporter*.

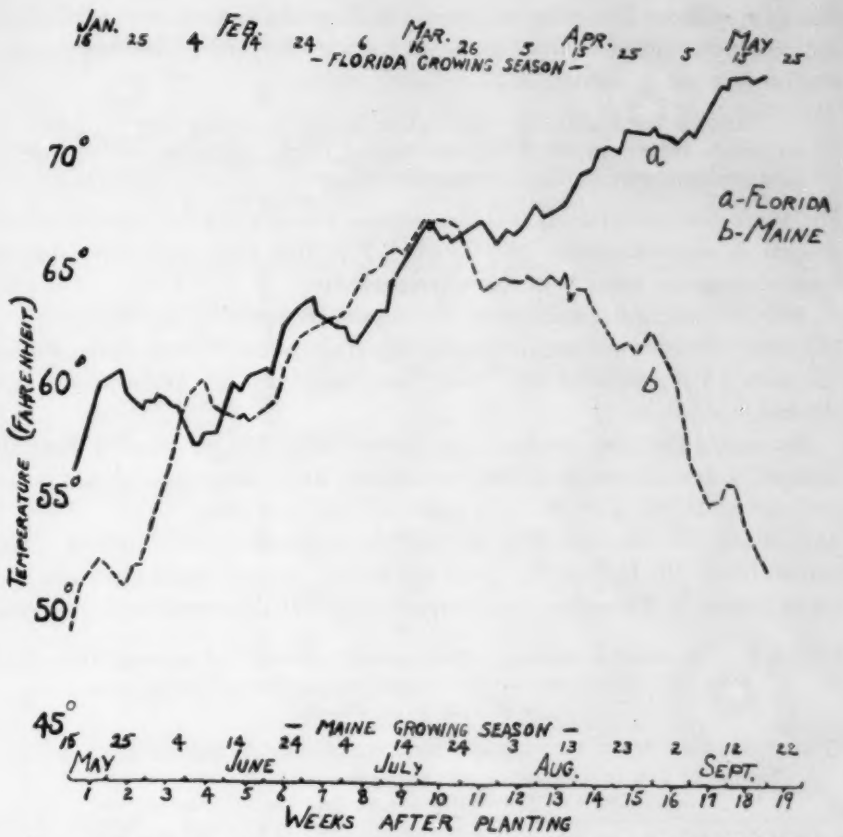

FIG. 3. Daily average mean temperatures during the ten-year periods 1917–1926 and 1916–1926[1] for the potato-growing seasons at Federal Point, Florida, and Aroostook Farm, Presque Isle, Maine, respectively.

Maine, although this is by no means the rule. Much tuber rot may be observed during certain seasons in Maine, but the amount of "mahogany rot" in Florida at time of harvesting is almost negligible except in a few instances of very early (Apr. 1–10) harvesting, where trouble may develop in transit to market.

By applying the known facts to existing conditions it is possible to explain, in a general way, these blight attacks in both sections. Melhus (12) clearly established that the optimal temperature for both the production of motile swarmspores (indirect germination) and infection is from 10 to 13° C. (50–56° F.), and the maximal is about 25° C. (77° F.). He demonstrated also that the optimal temperature for germ-tube or direct germina-

[1] Data for 1918 missing.

tion (*i.e.*, without liberating zoöspores) is about the same as the maximal for indirect germination. Direct germination was observed in his experimental studies, but not in nature, as he definitely states,

> "Indirect germination takes place in the morning dew and rain on potato foliage under field conditions. Direct germination was not observed to occur in the open on the foliage."

Melhus further ascertained that the optimal temperature for mycelial development is approximately 24° C. (75° F.), but that such development, though slower, is possible at lower temperatures.

Folsom, basing his opinion on the results of practical experience (4, p. 152) and on somewhat meager yearly reports of other investigators covering 12 years (5), concluded that the "cool weather" of Aroostook County checked late blight.

By examining the temperature curves (Fig. 1), we observe that the maximal temperatures in Maine throughout July, August, and early September are 21° C. (70° F.) and much of the time above 24° C. (75° F.). Also, it may be observed (Fig. 2) that the minimal temperatures in Maine remain above 10° C. (50° F.) from the middle of July until after the middle of August. Therefore, it appears doubtful if the weather of Aroostook

TABLE 4.—*Late blight in relation to frequencies of minimal and maximal temperatures from 1914 to 1928 during three months of the potato-growing season at Federal Point, Florida*

Year	Number of days in each month with the extremes of temperature indicated						Severity of late blight
	50° F. and below			70° F. and above			
	Feb.	Mar.	Apr.	Feb.	Mar.	Apr.	
1914	16	17	3	15	21	29	
1915	21	25	12	12	15	26	
1916	16	18	6	19	25	29	
1917	15	8	5	17	29	30	
1918	8	3	5	24	29	27	
1919	16	10	2	14	29	30	
1920	21	11	3	10	18	27	
1921	15	4	5	19	30	30	
1922	10	5	0	22	28	30	
1923	9	7	1	19	28	29	medium
1924	12	19	3	13	18	29	do
1925	10	12	6	21	26	29	severe
1926	16	15	11	20	23	30	do
1927	8	13	1	27	29	29	do
1928	17	14	13	16	25	27	none

TABLE 5.—*Late blight in relation to frequencies of minimal and maximal temperatures from 1913 to 1928 during three months of the potato-growing season at Presque Isle, Maine*

Year	Number of days in each month with the extremes of temperature indicated						Severity of late blight[1]
	50° F. and below			70° F. and above			
	July	Aug.	Sept.	July	Aug.	Sept.	
1913	4	16	26	26	27	10	medium
1914	17	22	25	26	22	18	do
1915	19	15	23	29	22	15	severe
1916	8	1	15	28	29	15	do
1917	5	5	29	28	25	11	do
1918	----	----	----	----	----	----	----------
1919	----[2]	15	27	----	20	6	10 per cent loss
1920	10	9	18	24	31	10	20 per cent do
1921	6	19	21	31	22	11	0.5 per cent do
1922	12	15	21	26	17	13	5 per cent do
1923	19	19	26	22	16	9	0 per cent do
1924	9	----	26	26	----	4	severe
1925	9	8	26	25	26	5	5 per cent loss
1926	10	17	29	25	22	4	7 per cent do
1927	7	15	23	30	25	9	25 per cent do
1928	12	8	26	27	24	6	severe

[1] 1913–1918 from Folsom (5).
1919–1927 from *Plant Disease Reporter*.
1928 from writer's personal observations.
[2] Indicating that no data are available.

County during the actual growing season is ever cool enough to greatly check late blight. In fact, the temperature more nearly approaches that reported by Melhus as the optimal for mycelial development of the fungus. The high percentage of moisture in the Maine fields, both in the form of dew and rain, also is favorable for the development of the fungus. Therefore, if the optimal temperature for development of the disease coincided with that for growth of the mycelium, it seems that little more could be desired to make conditions favorable for development of blight in the Maine fields. On the other hand, in view of the report of Melhus, it does not appear surprising to find epiphytotics of late blight in Maine fields after the middle of August when the minimal temperatures drop to about 10° C. although the maximal temperatures remain above 21° C. (70° F.) and that its failure to develop earlier in the season possibly is due to the higher minimal temperatures. The latter statement is supported by the investigations of Reddick under parallel conditions in New York (19). He demonstrated that, while

sporangial germination is possible either below or above 10° C. (50° F.), a temperature of 15.5° C. (60° F.) is too high for field infection of any appreciable amount. He presents experimental evidence to show that, while 21° C. (70° F.) is most favorable for swarmspore germination and penetration, the inhibiting factor for blight development under his conditions was not the failure of the swarmspores to germinate, but the failure of swarmspore production, as "the temperature of 60° (F.) was not low enough to initiate germination of the sporangia."

From the above conclusions we would expect that with sufficient moisture and high enough maximal temperatures during the day, the more frequently the minimal temperatures hover around 50° F. (10° C.) the more severe will be the epiphytotic. Table 5, based on climatological data for the 15-year period from 1913–1928 for Presque Isle, Maine, and giving the number of days during the growing season that the minimal temperatures were 50° F. or below, indicates that this theory is incorrect. It is observed there that such low temperatures in August are frequent, and yet the reports that blight was of but little consequence are also numerous. In the 15 years for which Presque Isle records are available, from 1 to 22 days in every July and August were found with minimal temperatures of 50° F. or less. There is then no reason to believe that blight was inhibited in Maine because the temperatures were too high. The average maximal temperature curve for Presque Isle (Fig. 1) indicates that ordinarily the temperatures were high enough for the vegetative growth of the blight organism (75° F.). Folsom and Bonde (5), giving average temperatures for 1913 to 1924, however, show that within that period a definite relationship between low temperatures in August and the absence of blight even with abundant moisture existed for but a few years. By comparing the average (mean) August temperatures from 1913 to 1928, with the occurrence of blight (Table 3), such an agreement is found for 1921, 1922, and 1923, where blight is not severe even with abundant moisture. This relationship is particularly apparent for 1921 and 1922 but not so pronounced for 1925 and 1926, where the injury was not exceedingly severe even though the temperatures were more favorable, especially in 1925. However, no such humidity-temperature relation is found for 1927. It is especially noteworthy that the August data for average temperatures and total rainfall for 1921 and 1927 are practically identical, and yet the injury was as severe in 1927 as it has been for many years, while in 1921 blight was almost negligible. It is true that in 15 years for which data are available 4 years (1916, 1917, 1920, and 1925) had both an average August temperature of over 65° F. (18° C.) and a severe epiphytotic of late blight (loss of 5 per cent or over), but it is also true that at least 6 of the 15 years had average August temperatures below 65° F. (mostly about 60–

TABLE 6.—*Comparative results obtained with samples of the same lots of healthy and Verticillium wilt-infected potatoes planted in Maine and Florida*

Seed stock	Aroostook Farm, Presque Isle, Maine						Hastings Laboratory, Hastings, Florida					
	No. of repl.	Stand	Wilt Per cent	Yield per acre (bushels)			No. of repl.	Stand	Wilt Per cent	Yield per acre (bushels)		
				Primes	Seconds	Culls				Primes	Seconds	Culls
			1926						*1926*			
Healthy	6	99	0.0	327.2	13.1	4.4	6	100	0.0	No yield records taken		
Wilt-infected	5	97	25.5	258.9	6.8	6.8	6	100	3.0[1]			
Decrease												
bushels				68.3	6.3	− 2.4						
per cent				20.9	48.1	−54.6						
			1927						*1927*			
Healthy	6	93.5	1.5	404.6	17.7	—	6	99	0.0	No yield records taken		
Wilt-infected	6	95.5	30.0	346.5	28.0	—	6	98	0.0			
Decrease												
bushels				58.1	−11.3							
per cent				14.3	64.8							
			1928						*1928*			
The experiment not conducted at Presque Isle this season							One bushel planted from wilt-infected seed stock					
							Two per cent of the plants indicated a slight vascular discoloration of the stem					
							No stem end browning in any of the tubers					

[1] Symptoms very slight.

61° F.) (15–16° C.), with a blight loss of 5 per cent or over. Again, since there are but 2 or 3 years where there is an actual relation between low average August temperature (approximately 60° F.) and no appreciable amount of blight, it is also true that during these 15 years, 1917 and 1920 are the only outstanding seasons where comparatively high August temperatures and precipitation records are associated with a severe blight attack, with the possible exception of 1928. It is possible that were records available for 100 years for Presque Isle, the conclusion that cool weather checks late blight could be reached, but at present such a conclusion seems unwarranted.

The fact is well established that the causal organism thrives under certain conditions of moisture, but, here, again, upon examining the climatological data (Table 3), no definite relation can be found and the indications are that in practically every year in Aroostook County there is sufficient moisture for the development of the fungus. The relation of high moisture and severe blight seems definite for 4 years where, in 1920 and 1928, the August rainfall was 3.3 inches or more and, in 1917 and 1927, over 5.0 inches. However, it is also to be noted that the rainfall for August, 1927, was the same as for August, 1921, and the losses from blight during those two years were 25 per cent, and ½ per cent, respectively, in spite of the fact that the mean temperatures for both years were identical (60.7 and 60.9° F.). No explanation for this difference has been advanced.

Attempts to show a relation between cool and wet, cool and dry, or warm and wet, and warm and dry weather and the occurrence of blight have not been very successful, as severe losses have been tabulated for Maine for all of these conditions, even in the few years for which records are available.

Upon returning now to the temperature data (Fig. 2), we observe that the minimal temperatures for Florida during the second month after planting are about the same as those in Maine during the similar portion of the growing season. During the third month after planting, however, the temperatures are more nearly the optimal for sporangial germination in Florida than in Maine. Blight is usually first observed in the Hastings belt any time after the first of March or even earlier, but the attack may be severe until late April, especially if the season is late. A study of the minimal temperatures (Fig. 2) will indicate why this is possible. Table 4, which gives the status of the minimal and maximal temperatures for the Florida area, indicates that the temperatures are both low enough for the initiation of an attack and high enough for mycelial development. A definite relation between climatic conditions and the severity of blight attacks cannot be established for this section because no definite records of epiphytotics are available except for the last few years.

Martin (11), in summarizing the work of others, points out clearly that the different investigators are not in accord as to the climatic conditions which are most favorable for the development of this disease. In presenting his own data, however, he indicates that definite correlations between temperatures and moistures and outbreaks of late blight are possible if the observations of many years are available. It is evident that the Florida and Maine data here presented are not sufficient and of long enough standing to answer definitely the questions which are constantly being presented. As Reddick (19) suggests,

> "It is unlikely that the existing records of weather are sufficiently detailed to give the minutiae necessary for the complete analysis of the situation, but rainfall and temperature records alone would probably give a more complete explanation of the conditions affecting the incidence of the disease than now exists."

These temperature records indicate the similarity between Aroostook County and Hastings conditions but do not always demonstrate to the investigator or to the grower what he may expect under the various existing climatic conditions. Only the records of many score more years will warrant more accurate predictions if such are to be made from past field experiences.

Early blight (*Alternaria solani* (*E. & M.*) *J. & G.*). Early blight causes about the same amount of damage in Florida as it does in Maine. Usually in both sections this trouble does not appear until the plants are approaching maturity. Occasionally the disease may appear earlier, but only in isolated instances where conditions have been particularly favorable for its development. In 1928 the disease was much more severe in Maine than during the 4 preceding years. This was also true for Florida, where the actual losses from this trouble alone were comparatively small. By comparing these facts with experimental evidence it is at once apparent that the field observations as made are about what could be expected. Rands (18) demonstrated definitely that the optimal temperature for both spore production and mycelial growth is about 26–28° C. (79–83° F.) but that growth is possible down to 1–3° C. (35° F.), and up to 37–45° C. (99–113° F.) or over. He further determined that, while heavy dews were an aid, abundant spore production took place only when such dews were supplemented by rainfall. Whetzel (28) likewise reports a serious epiphytotic of early blight in Bermuda following a season of warm, wet weather.

Examination of the temperature records (Figs. 1–3) shows that, with such a wide range between the maximal and minimal limits, there is no reason why this trouble should not develop in either section. Precipitation records (Tables 2–3) also indicate sufficient moisture in both sections, with a somewhat heavier rainfall at the end of the season in the South than at the end of the season in the North. It is reasonable to assume that the

greater precipitation abetted by the higher temperature toward maturity may be responsible for the slightly greater degree of severity of this disease in Florida, but the total percentage loss in either section as compared with that caused by late blight is small.

Rhizoctonosis (*Corticium vagum B. & C.*). Rhizoctonosis, perhaps better known as "black scurf" or the "little potato disease", much discussed as it has been, still presents a problem which is far from being solved. Richards' (20, 21) results demonstrated that this fungus may be parasitic through a range of from 9 to 30° C. (48–86° F.). He observed the greatest number of stem lesions at 12–21° C. (54–70° F.), with about 18° C. (64° F.) as the temperature where the most serious injury occurred.

Occasionally a small amount of injury may be observed in the very early (December) planted fields, but the percentage of "stem cankering" observed in the Florida territory was comparatively small, and there was seldom any killing of the tips of the stems, while the total number of hills affected with rhizoctonosis later in the season was always exceedingly small. In Maine, however, a rather high percentage of stem lesions and often considerable tip killing are observed and the number of plants manifesting typical rhizoctonosis symptoms later in the season is much larger than in Florida. The total loss in either section is not anywhere near 50 per cent, as Richards reports for Utah (22). In Maine the stem lesions frequently involve the entire stem below the surface of the ground and persist during the entire season. In the Florida potato belt the lesions seldom attain a length greater than from a quarter to a half inch and have not been observed to increase in size after the plants have become definitely established.

A comparison of the temperature curves (Figs. 1–3) indicates that in both sections the fungus should thrive during the 4- to 6-week period immediately after planting. With the knowledge that the actual damage occurs chiefly in the Maine fields, even though the temperatures are very similar in the early part of the growing season, it is evident that more extensive observations and experiments are necessary in both sections before a satisfactory explanation of the differences can be given. Soil-temperature data are not available. It is possible that these would not be so different in the two sections, and in that event an adequate explanation for this difference of behavior in the two sections must be sought elsewhere. One possibility is that the seed pieces in Florida are planted about 3 inches deep, on ridges, while in Maine the depth is almost twice as great and planting is practically on the level. Also, the soil is very sandy in the former section, and the surface layer, at the point where the fungus ordinarily is most destructive, may dry out much more quickly than in Maine where the soil is heavier and where planting is not on ridges.

Scab (*Actinomyces scabies* (*Thax.*) *Güssow*). Scab is considered of no commercial importance in the Florida potato section, and neither is it considered very destructive in Maine, although it is much more important in the latter section. The reason which Gillespie and Hurst (6) advance for the mild attacks in Maine is, ". . . an excellent correlation between the hydrogen-ion concentration (of soils) and occurrence of common potato scab." Their examination of a large number of soils from northern Maine revealed that those with a pH value of 5.2 or less rarely produced scabby potatoes. They found a difference in hydrogen-ion exponent between the Caribou and the Washburn loam, with a pH of 4.8 in the former and a much higher value in the latter, and the former producing scab-free potatoes, while those from the Washburn type were usually scabby.

Several score hydrogen-ion determinations were made on soils from different sections of the Hastings belt and they varied consistently in pH from 5.4 to 5.8. These figures are still within the lower limit of tolerance (pH 5.2) for scab development, but may be low enough for a certain amount of deterring effect. It is usually considered by the growers that the Hastings soils are too acid for the development of this disease. Whether or not other factors are involved remains to be determined. Millard and Burr (13) suggest the possibility of the existence of different species of the scab organism in soils of different reaction and texture, and ". . . under certain extreme conditions either of climate or soil, tubers are parasitized by one species or one cognate group of species to the exclusion of the others." Millard and Taylor (14) and Sanford (23) demonstrate the possibility of a certain balance between various parasitic and saprophytic fungi, and possibly bacteria, and that the particular rapid growth of the nonparasitic organisms, induced by the turning under of green cover crops, retards or inhibits the development of the scab pathogene, possibly through a lack of the proper nutrient materials, which results in the starvation of the parasitic type. Millard and Taylor suggest this possibility for soils with a comparatively high pH, while in other soils a high hydrogen-ion concentration may itself be the controlling factor. In the Hastings section a heavy growth of cowpeas and various native grasses is turned under annually, but the actual value of such green manuring, from the standpoint of inhibiting scab, is unknown. In Maine a similar procedure is followed, in part, where clover is turned under in the fall and potatoes are planted the following spring. It is reasonable to assume that such other hidden factors are involved, since the outstanding environmental conditions appear favorable for the growth of the causal organism. For example, Jones, McKinney and Fellows (8) have demonstrated that comparatively high temperatures are favorable for scab, and our temperature records (Table 1) indicate that, in general, the mild-

ness of this trouble is not due to unfavorable climatic conditions. Further intensive investigations are necessary in both sections to explain these differences.

Verticillium wilt (*Verticillium albo-atrum Reinke & Berth.*). In Maine a condition exists which is comparable with similar conditions reported from such other Northern States, particularly Oregon, where Verticillium wilt may at times be extremely serious. This trouble, which supposedly causes no decay of the tubers but causes a vascular discoloration of the stem and tubers and a great reduction in yield, is considered as existing only in the northern areas where cooler temperatures prevail. Chupp (2), however, mentions that "in New York where the summer temperatures are relatively low there are only occasional farms where the latter fungus is destructive," and thinks that the distribution is regional.

It has been well demonstrated that this fungus is disseminated through the seed potatoes and that it remains in the soil for many years. This being a fact, it is reasonable to expect that, other conditions being equal, the Florida soils should contain sufficient inoculum, considering the length of time that seed stock has been imported and planted from a region where this trouble is extant. Verticillium wilt has been seldom observed in Florida, even though the seed stock frequently comes from Maine fields where rather high percentages were present.

In order to gain more definite information regarding this trouble 2- to 3-bushel samples of tubers were selected during several consecutive seasons in Maine[3] from hills which had been killed by this organism. The tubers were carefully examined and each one was rejected which did not have the typical stem-end browning.

These samples were divided into two lots, and during the following season one lot was planted in Florida and the other in Maine. In each plot the sample was compared directly in alternate rows in several replications with tubers taken from wilt-free hills. The percentage of hills exhibiting symptoms of the disease was determined each year. These figures are given in table 6. Practically no wilt was observed on these plantings in Florida at any time during the season, while comparatively high percentages of wilt occurred in the same stock (Table 6) when planted in Maine. Comparative yield data were obtained in Maine but not in Florida because in the latter section the plants both from clean and diseased tubers showed no differences and apparently all were healthy. Why this disease is severe in one of these sections and not in the other is unexplained. Edson and Shapovalov (3) when comparing in culture two strains of *V. albo-atrum* discovered that the one from Maine shows a much better adaptation to

[3] This experiment was conducted in cooperation with Doctor E. S. Schultz, Senior Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture.

lower temperatures while the strain from West Virginia exhibits a better adaptation to higher temperatures, the former giving no growth and the latter giving fair growth at 30° C. (86° F.). Reasoning from the results of these investigators, the conclusion would be reached that, since the temperatures are so nearly alike in both sections for part of the growing season and since the maximal temperature for the growth of this fungus is at no time approached until the latter part of the season, Florida fields should suffer severe losses through Verticillium wilt. It is possible that the increase in temperature in this southern area may aid in retarding the development of this fungus, but it is reasonable to believe that in some sections of the potato area and in some seasons at least mild symptoms should be observed. Such conclusions have not been confirmed by the observations of the writer during the last 6 years. Again, soil temperatures are not available for both sections, but the air temperatures given above serve as an index of what the soil temperatures may be, and it is possible that other factors are also involved.

BACTERIAL DISEASES

Blackleg (*Bacillus atrosepticus van Hall*). The blackleg situation presents another interesting problem. This disease was much discussed and investigated a decade or so ago. Morse (15, 16, 17), and others, later, found that consistent treatment and careful selection of the seed stock gave good commercial control, and, until several years ago, this disease was considered as becoming less important. Two years ago, however, it gained a fresh momentum when, in Maine and other northern potato-producing States, very high percentages of this trouble occurred. In Maine high percentages were found also in fields planted from seed stock in which, the preceding year, no trace of this disease had been observed. Several of the best growers in Aroostook, who had carefully hand picked their seed potatoes from tuber unit material and had treated them with the highly recommended hot-formaldehyde method, were not granted any immunity but suffered fully as great injury as others who followed the ordinary routine methods of planting average seed potatoes.

Various investigators at once began to theorize and search for satisfactory explanations, only to be baffled a second year with a recurrence of similar high percentages. In the Maine fields diseased stalks were observed frequently which showed typical symptoms of this disease on parts above ground, while on the remainder of the stem and on the seed piece there was no decay or discoloration of any sort. This suggests the possibility of insect transmission in the Maine fields, as was found to be the case in Minnesota by Leach (10). The problem is by no means solved, but the fact that the seed-corn maggot (*Hylemyia cilicrura* Bond.) can be responsible has

been confirmed recently by Bonde (1). Whether other insects or other factors are concerned has not been demonstrated.

During these several years of high percentages of blackleg infection in Maine the Florida fields were carefully observed, but nowhere has the amount increased perceptibly over previous years. Only a slight trace of this disease has been evident in Florida for the last 6 years.

In addition to field observations for the past several seasons, tubers were carefully selected from blackleg hills of the Spaulding Rose No. 4 variety in Maine fields and were planted in Florida. Upon arrival in Florida the tubers always showed much rot because only those were selected in Maine which showed unmistakable symptoms of the disease. Precautions were taken to plant only those seed pieces which were partly decayed or which had been covered with the decayed material from other tubers. Frequently, the potatoes were split lengthwise to insure the presence of a decayed portion on each seed piece. In 1928 but 3 hills manifested the disease in a total of 39 tuber unit samples of 4 hills each, and each one of these diseased hills was in a different unit. In 1927 similar results were obtained and not over 3 to 4 per cent blackleg hills were observed in the rows planted with such diseased material. Such failure to produce the disease by planting diseased tubers has been reported by other investigators and has not been explained satisfactorily. Again, the possibility of insect dissemination or absence of such spread is suggested. The seed-corn maggot (*Pegomyia fusciceps* Zett.) was reported from Florida in 1905 (26).

Bacterial wilt (*Bact. solanacearum EFS.*). Bacterial wilt, bacterial blight, and southern brown rot are common names of this disease. According to Smith (27), who isolated the causal organism in 1896, the disease occurs in the United States from Maryland and New Jersey south to Cuba and Porto Rico. The writer has never observed the disease in Maine, and as far as can be ascertained it has never been reported from there.

The disease apparently is favored by high temperatures and appears to be more or less regional, similar in that respect to Verticillium wilt as described by Chupp (2). It is found regularly in some fields year after year, while in other near-by fields only traces have been observed from time to time. The growers have learned from practical experience that early planting and, consequently, early harvesting provide a partial escape from great losses. It is assumed that slightly lower temperature is the controlling factor in these instances. It is not uncommon to observe symptoms of this disease in newly cleared land in Florida and on ground on which solanaceous plants have not been observed. Much experimental work has never been reported, and the total percentage losses, while not extreme, are still large enough to warrant careful investigational work.

Virus diseases (causes not determined). The losses caused by the various degeneration diseases are about as large in the potato fields of Florida as in the Spaulding Rose No. 4 variety in Maine, the percentages being comparatively low in both States. It is to be remembered that the Spaulding Rose No. 4 is about the only variety grown in the Florida potato belt. Since this particular variety apparently is not so susceptible to mosaic as either Bliss Triumph or Green Mountain (7), commercially, the Hastings area has smaller losses through these troubles than Aroostook County. There the relative acreage of other varieties is much greater than that of the Spaulding Rose No. 4. Virus diseases, as they have been observed in both potato sections, will be discussed in a later paper.

SUMMARY

Detailed climatological data, consisting of daily maximal, minimal, and mean temperatures, based on 10 years observations in both the Hastings, Florida, and the Aroostook County, Maine, sections, together with monthly averages of temperature and rainfall, are given and the relations of these facts compared with the occurrence of such diseases as late blight, early blight, rhizoctonosis, scab, and blackleg, which are common to both sections, are pointed out.

Verticillium wilt is shown to be very severe in Maine during certain seasons and negligible in Florida, while bacterial blight is prevalent in Florida and unknown in Maine.

The percentage of virus disease is about the same in the Florida potato section, where the Spaulding Rose No. 4 is the only variety grown, as in the Spaulding fields of Aroostook County.

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STUDIES UPON A BACTERIOPHAGE SPECIFIC FOR PSEUDOMONAS TUMEFACIENS

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Although bacteriophagy in its relation to human and animal pathogenes has been the subject of intensive investigation, little is known as to the occurrence of such lytic principles affecting phytobacteria. In the light of previous work by d'Herelle (8), Cruz (4), Gratia (6), Hauduroy (7), and others dealing with human and animal pathogenes, such considerations in phytopathology as the cause of loss of virulence in bacterial culture, the inactivation or death of bacteria in soil or overgrowths, the occurrence of nonvirulent strains of pathogenes within the host may well be investigated as to their relation to bacteriophagy.

Probably Mallman and Hemstreet (13) were the first to isolate an inhibitive agent from plant material. From a decaying cabbage affected with a fluorescent soft-rot bacterium, they obtained a lytic agent active against the causal rot organism as well as *Bacillus carotovorus* Jones. They also showed that at dilutions of 10^{-17} the bacteriophage would inhibit growth of the soft-rot organism in broth cultures.

At about the same time Gerretsen and Sack at Groningen and Gryns and Söhngen at Wageningen worked independently upon the bacteriophage of the legume nodule organism, *B. radicicola* Beij. Later these investigators (5), in collaboration, published the results of their work on the isolation of a bacteriophage from the nodules, roots, and stems of bean, clover, lupine, and Seradella plants. These investigators, apparently, were the first to demonstrate strain specificity of a bacteriophage from plants. The lytic principle isolated from bean was active against the strain of nodule bacteria from this plant and clover. The bacteriophage from clover nodules affected the organism from clover, but not from lupine nodules. They also isolated a bacteriophage from a culture of the Seradella nodule organism sent them from another laboratory. However, this lytic principle was not active against their own isolation of *B. radicicola* from the same host. Hitchner (10) also obtained a bacteriophage from the nodules of red clover active only against the strain of *B. radicicola* from clover.

[1] The writers wish to acknowledge their indebtedness to Dr. I. E. Melhus for helpful criticisms and suggestions during the course of these investigations and in the preparation of the manuscript.

These studies have been carried out at Iowa State College in connection with the crown-gall project in which the U. S. Department of Agriculture, the Crop Protection Institute of the National Research Council, University of Wisconsin, and Iowa State College are cooperating.

Anderson (1), working with *Ps. pruni* E. F. S., isolated a bacteriophage from the soil beneath a peach tree previously infected with the leaf-spot disease, but did not obtain a lytic agent from old infected leaves, although only one trial was made. The addition of the bacteriophage at high dilutions to agar-plate colonies produced typical plaques, although secondary cultures appeared in these areas after several days.

Coons and Kotila (3) isolated a polyvalent bacteriophage from a carrot rotted by *B. carotovorus*. In this case the lytic principle was active against *B. atrosepticus* van Hall and *Ps. tumefaciens* Smith and Town., as well as the soft-rot organism. After standing five and one-half months the lytic filtrate (ninth passage), although still effective against all the organisms tested, was not capable of causing clearing except in cultures of *B. carotovorus*.

Israilsky (11) later obtained from a crown gall on sugar beet a bacteriophage effective against *Ps. tumefaciens*. From the same gall he obtained two cultures (out of 11 isolations) which were resistant to the lytic principle. Similar resistant strains of the crown-gall organism were isolated from broth cultures to which the bacteriophage had been added in dilutions of 10^{-10} and 10^{-11}.[2] These strains also remained resistant at higher concentrations of the bacteriophage. He was unable, however, to isolate the lytic principle from a pure culture of *Ps. tumefaciens*.

Brown and Quirk (2) recently reported the isolation of a bacteriophage from tumors on *Ricinus communis* L. and sugar beets. In certain cases filtrates from the Ricinus tumors inhibited growth for 20 days when added to young cultures of the crown-gall pathogene. Complete lysis was not obtained in any of the treated cultures, although bizarre forms of the organism were demonstrated. When cultures of *Ps. tumefaciens* treated with high dilutions of the filtrates from tumors and rotted carrots were inoculated into Ricinus plants, galls were induced more rapidly than in the controls and were larger. The addition of such filtrates to young cultures of *Ps. tumefaciens* also stimulated growth. These investigators were unable to demonstrate the presence of an infectious filterable form of the crown-gall pathogene.

The writers (14) previously reported the results of experiments showing the lytic action of two bacteriophages isolated respectively from a crown gall on sugar beet and from a pure culture of *Ps. tumefaciens* (raspberry isolation). It was shown also that the bacteriophage was specific in its lytic action against certain plant pathogenes and isolations of the crown-gall organism in that it affected only the strain of *Ps. tumefaciens* from which it

[2] As a matter of convenience the negative exponent is used to express dilutions. Thus the expression 10^{-2} denotes a distribution of 1–100, 10^{-6} a dilution of 1–1,000,000, etc.

was isolated. These studies have been extended and the results are given in more detail in the present paper.

METHODS AND MATERIALS

The lytic principles (bacteriophages) were obtained from five sources as follows: I, a culture of *Ps. tumefaciens* in unsterilized soil no longer infectious; II, a crown gall on *Rumex crispus* L. produced by artificial inoculation with the Rumex strain of *Ps. tumefaciens*; III, a sugar-beet gall induced by artificial infection with the raspberry isolation of *Ps. tumefaciens*; IV, a culture of *Ps. tumefaciens* in sterilized soil no longer infectious; and V, a virulent culture of *Ps. tumefaciens* isolated from a raspberry gall in 1923. In this connection it should be noted that the soil cultures were originally inoculated January 12, 1926, with the raspberry isolation of the crown-gall organism. The sugar-beet gall was induced by inoculation with a subculture of the organism from which lytic principle V was obtained. Thus bacteriophages I, III, and IV were obtained from inoculation with subcultures of the same isolation of *Ps. tumefaciens.*

In obtaining lytic principles I and IV, 10 grams of the soil were placed in 30 cc. of neutral peptone-dextrose broth and incubated at room temperature for thirty hours. The cultures were then filtered through paper and the filtrate passed through a Berkefield W filter. Four days later 20 cc. of a 24-hour broth culture of *Ps. tumefaciens* was added to the clear filtrate and after standing three days was filtered as before. This procedure of adding cultures of the organisms and filtering was repeated at intervals of four to seven days or longer.

Lytic principles II and III were obtained by filtration from cultures made by adding 20 grams of the finely ground galls from sugar beet and *R. crispus* to 50 cc. of neutral peptone-dextrose broth. The cultures were incubated 24 hours at room temperature, filtered through filter paper, and then through Berkefeld filters. Subsequent additions of young broth cultures of *Ps. tumefaciens* to the filtrates and filtrations were made as given above. Lytic principle V was obtained from a seven-day-old neutral-broth culture of *Ps. tumefaciens* isolated from a raspberry gall in 1923 and carried as a stock culture for inoculation studies. The method employed was a series of filtrations through Berkefeld filters with additions at irregular intervals of 10 cc. of a 24-hour broth culture to the sterile filtrate. At different times during the progress of these successive additions and filtrations, tests were made of the presence of a lytic principle in the filtrates.

EARLY STUDIES OF THE BACTERIOPHAGE

After four successive filtrations one cc. of the filtrates I to V, inclusive, was added to 9 cc. of a 24-hour-old broth culture of the raspberry strain (R)

of *Ps. tumefaciens*. This procedure was repeated, using different dilutions of the filtrate so as to make the final dilution of the lytic principle 10^{-2} to 10^{-6}, inclusive. Readings taken at the end of seven days showed growth in all the dilutions of lytic filtrates I, II, and IV but in none of the dilutions of filtrates III and V.

After seven filtrations, again one cc. of each of the filtrates I to V, inclusive, was added to sterile broth, making dilutions of 10^{-2} to 10^{-6}, inclusive. To each of the diluted filtrates was added one cc. of a 48-hour broth culture of *Ps. tumefaciens* and records taken at the end of seven days. The results of this trial are given in table 1.

TABLE 1.—*The effect of lytic filtrates in different dilutions upon the growth of Pseudomonas tumefaciens*[a]

Lytic filtrate	Dilutions of filtrate and growth reactions					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Check
I	4	4	4	4	4	4
II	4	4	4	4	4	4
III	0	0	0	0	0	4
IV	0	0	0	0	0	4
V	0	0	0	0	0	4

[a] Growth is indicated as follows:
0 = No growth, broth clear.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth with pellicle formation.

Although the filtrates I and II showed growth at dilutions of 10^{-2} to 10^{-6}, inclusive, there was a certain amount of the lytic agent present in the undiluted stock filtrate. The addition of 10 cc. of a 24-hour culture of *Ps. tumefaciens* to 30 cc. of the filtrates above mentioned failed to show clouding of the culture until five days had elapsed and then only a slight turbidity was evident.

To test further the lytic action of the five stock filtrates, inoculations were made into tomato plants. For this purpose 10 cc. of a culture of *Ps. tumefaciens* was added to 30 cc. of the stock filtrate and allowed to stand two days. At this time there was no visible growth in the filtrates. Inoculations were made by needle prick into the tips of succulent young tomato plants. No galls developed from the filtrate inoculations while the checks inoculated with *Ps. tumefaciens* showed typical galls twenty-four days later.

EFFECT ON PSEUDOMONAS TUMEFACIENS OF CONTACT WITH LYTIC FILTRATES OF DIFFERENT DILUTIONS AND FOR DIFFERENT LENGTHS OF TIME

One cc. of a 24-hour broth culture of *Ps. tumefaciens* was added to one, two, three, four, and five cubic centimeters of the stock lytic principle III. At this time the lytic filtrate had undergone fourteen series of additions and filtrations and had been standing two months since the last series. The above mixtures, after standing for different periods of time ranging from three to 60 hours, were used in inoculations of young tomato plants.

Two plants were inoculated by needle with each mixture, ten punctures being made in each stem. Results of these trials 30 days after inoculation are presented in table 2. Table 2 shows that inoculations with all the mixtures at the end of three hours produced galls, showing that *Ps. tumefaciens* had not been inactivated up to that time. At the end of six hours, however, at a concentration of one part of the suspension of the organism to two parts lytic filtrate, and greater, no galls were produced. At concentrations of one to four and one to five, slight smooth swellings developed at the edge of the punctures, but attempts at reisolation using bile agar failed to recover the pathogenes from these swellings. After nine hours and until the termination of the experiment at 60 hours no galls were produced by inoculation with the mixtures at any of the concentrations. In all cases the lytic filtrate

TABLE 2.—*Results of inoculation into tomato of a mixture of one cc. of a Pseudomonas tumefaciens suspension with different quantities of lytic filtrate III after reacting for various lengths of time*[a]

Contact periods hours	Proportions of suspension to lytic filtrate III					Checks	
	1–1	1–2	1–3	1–4	1–5	*Ps. tumefaciens* 1–0	Lytic filtrate III 1–0
3	2[b]	1	1	1	0	2	0
6	1[b]	0	0	1	0	2	0
9	0	0	0	0	0	2	0
12	0	0	0	0	0	2	0
24	0	0	0	0	0	2	0
48	0	0	0	0	0	2	0
60	0	0	0	0	0	2	0

[a] The results of inoculations are indicated as follows:
0 = No galls formed.
1 = Small swelling, not typical galls.
2 = Typical crown gall.

[b] *Ps. tumefaciens* was reisolated from the gall.

alone and *Ps. tumefaciens* were inoculated into tomato plants as checks. In these trials, the lytic filtrate alone failed to produce galls, and *Ps. tumefaciens* consistently produced typical crown galls.

It is interesting to note in this connection that Israilsky (12) showed that rape plants inoculated with a culture of *Ps. tumefaciens* treated with bacteriophage showed a material reduction in the percentage affected with crown gall compared with those inoculated with untreated cultures. He also soaked rape seed two hours in the lytic filtrate at dilutions of 1 to 10 and 1 to 100, to which *Ps. tumefaciens* had been added. In each case there was a decrease in the number of plants with crown gall after treating the seed with the bacteriophaged cultures as compared with untreated cultures.

Further attempts were made to prevent crown-gall infection by the use of bacteriophage. In this experiment one drop of the undiluted lytic filtrate V was injected into the succulent tips of a series of young tomato plants and the point of injection marked with India ink. These trials were made in triplicate. At intervals of two days, up to two weeks, beginning two hours after the injection, *Ps. tumefaciens* was introduced into the wound made by the hypodermic needle. In no case was crown-gall infection prevented. Why the bacteriophage after two hours in the plant did not inhibit the growth of *Ps. tumefaciens* is not clear. It is possible that the acidity of the sap of the tomato plant may have inactivated the lytic agent since it is well known that the bacteriophage is most active in media with neutral or slightly alkaline reactions.

INCREASE IN POTENCY OF THE LYTIC PRINCIPLE AFTER SUCCESSIVE FILTRATIONS

At varying intervals the potency of the lytic principles was tested by making dilutions in alkaline or neutral peptone-dextrose broth and inoculating the tubes with the crown-gall organism. For this purpose filtrate III after three and seven filtrations was employed. The filtrate was made up to dilutions of 10^{-2} to 10^{-6} by the addition of one cc. of the lytic filtrate to 9 cc. of alkaline broth. To each 10 cc. of the diluted filtrate was added one drop of a 24-hour-broth culture of *Ps. tumefaciens* (original raspberry isolation). The results of these trials are shown in table 3.

While the filtrate after three filtrations did not entirely prevent growth, there was distinct evidence of partial inhibition after seven days. At a dilution of 10^{-2} there was only a very faint turbidity of the culture which became more pronounced as the dilution became greater. However, in none of the dilutions was the culture so turbid as in the untreated check culture of *Ps. tumefaciens*. After seven filtrations, the filtrate at all dilutions remained clear seven days after inoculation with *Ps. tumefaciens*. The writers (14) have already shown that after 17 passages through Berkefeld

TABLE 3.—*Increase in potency of the lytic principle after successive filtrations*[a]

Filtrate III	Dilution of filtrate and growth reaction					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Check
Third filtration	1–	1	2	2	2	4
Seventh filtration	0	0	0	0	0	4

[a] Growth is indicated as follows:
0 = No growth, broth clear.
1– = Very faint haze but less growth than 1.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth with pellicle formation.

filters, lytic filtrates III and V at dilutions 10^{-10} and 10^{-14} caused lysis of 24-hour-old cultures of the crown-gall organism.

Further trials were made of the potency of the lytic filtrates III and V after 19 passages, respectively, through Berkefeld W filters. Each series of trials was repeated three times with the same results. In these trials the dilutions of the lytic filtrates were made by adding 0.5 cc. of the liquid containing the bacteriophage to 5 cc. of neutral peptone-dextrose broth. With the same pipette 0.5 cc. of the first dilution (1–10) was withdrawn and added to the second tube until five dilutions were made. A fresh sterile pipette was then used in making the next five dilutions and so on for the entire series. With this method of making the dilutions it is possible that a certain amount of the lytic principle may have been carried over on the walls of the pipette, although it was emptied at each dilution and thoroughly flamed to steaming before making the next dilution. The reaction of the lytic principles upon *Ps. tumefaciens* at dilutions greater than 10^{-30} is given at intervals of 10 because in all cases the growth of the organism was the same as in the check tubes of untreated broth. There was no growth in the tubes containing dilutions of the filtrate at 10^{-1} and 10^{-10}, hence in the table these data are omitted. The data from these tests are given in table 4.

From this table it is seen that at dilutions of 10^{-1} to 10^{-21} both lytic filtrates inhibited growth of *Ps. tumefaciens* for 120 hours following the addition of the inoculum. After 144 hours turbidity occurred to the extent of a faint haze in lytic principle III at a dilution of 10^{-21}. At 168 hours the turbidity had increased to the extent that the medium was slightly cloudy. At this dilution there was no evidence of growth of *Ps. tumefaciens* in the tubes containing lytic principle V. At dilutions of 10^{-22} very slight

TABLE 4.—*The effect of lytic filtrates V and III at various dilutions upon growth of Pseudomonas tumefaciens*[a]

Dilution of filtrate	Lytic filtrate V — Growth of culture Hours							Dilution of filtrate	Lytic filtrate III — Growth of culture Hours						
	24	48	72	96	120	144	168		24	48	72	96	120	144	168
10^{-20}	0	0	0	0	0	0	0	10^{-20}	1–	0	0	0	0	0	0
10^{-21}	1–	0	0	0	0	0	0	10^{-21}	1–	0	0	0	0	1–	1–
10^{-22}	1–	0	1–	1–	1–	1	1	10^{-22}	1–	0	0	1–	1–	1	2
10^{-23}	1–	0	1–	1–	1–	2	3	10^{-23}	1–	0	0	1–	1–	1	3
10^{-24}	1–	0	1–	1–	1–	2	2	10^{-24}	1–	0	0	0	1–	1	3
10^{-25}	1–	0	1–	1–	2	2	3	10^{-25}	1–	0	0	1–	1–	1	3
10^{-26}	1–	0	1–	1–	2	3	4	10^{-26}	1–	0	0	1–	1–	1	3
10^{-27}	1–	2	2	3	4	4	4	10^{-27}	1–	0	1–	1	2	2	3
10^{-28}	1–	2	2	3	4	4	4	10^{-28}	1	0	1–	1	2	3	4
10^{-29}	1–	2	2	3	4	4	4	10^{-29}	1	2	2	2	3	4	4
10^{-30}	1	2	2	3	4	4	4	10^{-30}	1	2	3	2	3	4	4
10^{-40}	1	3	3	3	4	4	4	10^{-40}	1	3	3	3	4	4	4
10^{-50}	1	4	4	4	4	4	4	10^{-50}	1	3	3	3	4	4	4
Check *Ps. tumefaciens*	1	4	4	4	4	4	4	Check *Ps. tumefaciens*	1	4	4	4	4	4	4
Check filtrate V	0	0	0	0	0	0	0	Check filtrate III	0	0	0	0	0	0	0

[a] Growth is indicated as follows:

0 = Clear liquid with few climps of agglutinated bacteria.
1– = Very faint haze but less growth than 1.
1 = Slight turbidity.
2 = Medium turbidity.
3 = Moderately heavy turbidity.
4 = Heavy turbidity and pellicle formation.

turbidity showed in the tubes of both lytic filtrates after 24 haurs. However, the cultures cleared by the end of 48 hours. After 72 hours filtrate V at dilutions of 10^{-22} to 10^{-26} showed a very faint turbidity with a few clumps of the agglutinated bacteria. This condition remained unchanged at the end of 120 hours.

With the lytic filtrate III slight turbidity was also seen in the tubes at dilutions of 10^{-22} to 10^{-26} after 24 hours. The cultures cleared after 48 hours and remained unchanged 72 hours after inoculation. At the end of 120 hours there was also slight turbidity of the culture with a few clumps of agglutinated bacteria.

In both series of cultures at dilutions of 10^{-22} to 10^{-26} turbidity increased after 144 hours until at the termination of the experiment there was practically the same amount of growth as in the check tubes. At dilutions of 10^{-27} to 10^{-50} no lytic action was shown by either filtrate, the growth of the cultures at each reading being identical with that of the untreated check tubes.

At the higher dilutions 10^{-21} to 10^{-25}, inclusive, it seems possible that, in view of the corpuscular nature of the bacteriophage, the preponderance of *Ps. tumefaciens* over the quantity of the lytic agent might account for the continued growth of some of the bacteria. After standing 144 hours poured plates were made of the cultures in lytic filtrate V at a dilution of 10^{-22} by adding two cc. of the filtrate to 30 cc. of crystal violet bile agar. After six days the three plates showed only 47 colonies of the crown-gall organism. In the plates from the untreated check tubes, using two three-millimeter loops of the inoculum to the same amount of agar, the colonies of *Ps. tumefaciens* were too numerous to count.

INACTIVATION OF THE LYTIC AGENT IN BILE AGAR CULTURES

In attempts to demonstrate the formation of plaques in cultures of *Ps. tumefaciens* by lytic principles III and V, the organisms was streaked upon agar poured plates and a drop of the lytic principle placed in the center of the streak. As bile crystal violet agar has been found to be an excellent medium for the growth of the crown-gall organism, this medium was employed in the above tests. However, it was found that the lytic filtrate when applied to the organism on this medium failed to form plaques, that is, failed to lyse *Ps. tumefaciens.*

Having previously demonstrated that the lytic filtrate of this passage prevented growth of the pathogene in broth cultures, further trials were made with streaks of the organism on plates of neutral potato-dextrose and neutral bile crystal violet agar. The plates were examined two days after streaking and the application of the lytic principle. As checks the streaks

of the organism on agar plates were not treated with the lytic principle. In every case the application of a drop of the lytic principle to the streak culture of the organism on neutral potato-dextrose agar caused lysis, while in the bile agar plates there was no lytic action. These results are in accord with those of d'Hérelle (9) who showed that bile exerts an inhibitory action upon the Shiga bacillus bacteriophage but does not cause its destruction.

VARIATION IN SUSCEPTIBILITY OF PSEUDOMONAS TUMEFACIENS TO THE ACTION OF THE BACTERIOPHAGE

The plate cultures made from lytic filtrate V at a high dilution, to which *Ps. tumefaciens* had been added, indicated that certain individual bacteria within the culture might be more resistant than others to the action of the lytic agent. The phenomenon of resistance and susceptibility to bacteriophage has been demonstrated by d'Hérelle (9), Gerretsen, Gryn, Sack, and Söhngen (5), Coons and Kotila (3), and others. Attempts, therefore, were made to determine whether there was any morphological difference between the colonies of *Ps. tumefaciens* which were resistant and those susceptible to the lytic agent. Using bile agar, dilution plates were made from certain lytic filtrate cultures which showed no turbidity at dilutions of 10^{-3} to 10^{-5} after having been exposed to the bacteriophage for seven days.

Only a few colonies came up on the plates and these were of the rough type, i.e., showing striations of the surface growth and with serrate margins. Plate cultures were also made from the lytic filtrate at dilutions of 10^{-17}, 10^{-15}, and 10^{-30}, which showed moderate turbidity after seven days. There was an abundant colony development on all these plates. The colonies in these cases were typical of the smooth type with serrate margins. Subcultures were made from the smooth and rough type colonies and these, after 24 hours growth in broth, were used to inoculate young tomato plants and also lytic filtrates III and V at dilutions of 10^{-1} to 10^{-20}, inclusive. There was no inhibitive action shown by the filtrates and galls developed on the inoculated plants. These results suggest that certain of the bacteria may be resistant to the action of the bacteriophage as shown by d'Hérelle (9) and, in such cases, growth is inhibited but complete lysis does not take place.

TEMPERATURE RELATIONS

Coons and Kotila (3), working with a polyvalent bacteriophage from decaying carrot, showed that incubation at 36.1° C. for six days greatly decreased the lytic action against *B. carotovorus*. Mallman and Hemstreet (13) showed that their inhibitory substance, isolated from a decaying cabbage, was killed by exposure of 30 minutes to a temperature of 63° C.

Tests were made to determine the effect of high temperature upon bacteriophage V. In these tests, three tubes containing 2 cc. of lytic principle diluted one to one with alkaline broth were suspended in a water bath with a fourth broth tube in which was placed a thermometer. By constant stirring, the temperature was held at a maximum variation of one degree. After the liquid in the thermometer tube had reached the required temperature, it was held for ten minutes. Usually only two minutes were necessary to raise the temperature in the tubes from 25° C. to that required. After heating the tubes containing the lytic agent they were cooled at 25° C. and 0.1 cc. of a 24-hour broth culture of *Ps. tumefaciens* added. Preliminary tests were made at temperatures of 45, 50, 55, 60, 65, 70, and 75° C. After cooling, the lytic principle was diluted to 10^{-2} in sterile broth and one drop of a 24-hour broth culture of *Ps. tumefaciens* added. Readings were made each 24 hours until 96 hours had elapsed. The diluted, heated filtrates at all temperatures showed very slight clouding of the liquid after 48 hours. At this time a few clumps of the agglutinated bacteria were also present as a precipitate in the tubes. However, after 72 hours the turbidity disappeared in every case with an increase in the masses of agglutinated organ-

TABLE 5.—*Effect upon lytic filtrate V of heating for ten minutes at different temperatures*[a]

Culture method	Temperature; degrees C.								Check not heated
	60	70	75	80	85	90	95	98	
A	0	0	0	1[b]	4	4	4	4	4
B	0	0	0	1[c]	4	4	4	4	4
C	0	0	0	1[d]	4	4	4	4	4

[a] Culture methods A, B, and C refer to the methods 1, 2, and 3, as given in the text immediately preceding the table. Growth is indicated as follows:

[b] One tube showed no growth; one tube showed slight turbidity with masses of agglutinated bacteria and one tube with abundant growth.

[c] Two tubes showed no growth; one tube showed slight turbidity with a few precipitated clumps of bacteria.

[d] Two tubes showed no growth; one tube showed slight turbidity with clumping of the bacteria.

0 = No growth.
1 = Very slight growth.
2 = Slight growth.
3 = Moderate growth.
4 = Abundant growth with pellicle formation.

isms. The results of this test showed that a temperature of 75° C. for 10 minutes did not inactivate the lytic principle; therefore, further trials at higher temperatures were made. The following trials at temperatures of 60, 70, 75, 80, 85, 90, 95, and 98° C. were made in triplicate, employing the same method as given previously.

The effect of heating upon the lytic principle was tested in three sets of cultures by the following methods: (1) One drop of the heated lytic principle was added to a 10 cc. 24-hour-old broth culture of *Ps. tumefaciens*; (2) one drop of the lytic principle and one drop of inoculum were added to 9 cc. of sterile broth; (3) 2 cc. of the lytic principle were added to an equal volume of a 24-hour-broth culture of *Ps. tumefaciens*. Final readings of the cultures were made after 168 hours.

The summarized results of the three trials are presented in table 5.

From the data presented in table 5 it is evident that the lytic principle was completely inactivated by heating for 10 minutes at 85° C. At a temperature of 80° C. for 10 minutes, although the lytic principle was not able to prevent growth of the crown-gall organism in every tube (see footnote of table), there was still evidence of inhibition to bacterial growth. The results of these tests show that the temperature causing inactivation of the lytic principle V lies between 80° and 85° C.

Previous experiments having shown that lytic filtrate V in high dilutions would inhibit the growth of *Ps. tumefaciens*, the question arose as to whether or not the filtrates would be effective against phytopathogenes other than the crown-gall organism. It was also of interest to know whether this filtrate would likewise inhibit the growth of *Ps. tumefaciens* isolated from other hosts or from the same host at various times. Although the valency of lytic principles had been determined by other investigators, no tests employing the same pathogene from numerous isolations had been made.

SPECIFIC ACTION OF THE LYTIC PRINCIPLE

Coons and Kotila (3) and Mallman and Hemstreet (13) showed that the bacteriophages which they isolated from decaying carrot and cabbage, respectively, were polyvalent. Gerretsen, Gryn, Sack, and Söhngen (5) found the bacteriophage from legume nodules monovalent and specific for certain strains of *B. radicicola*. Tests, therefore, were made to determine the valency of lytic principle V and the specificity of its action upon *Ps. tumefaciens* isolated at different times from various hosts.

After fourteen successive passages through a Berkefeld W filter, the lytic filtrate V in dilutions of 10^{-1} to 10^{-9}, inclusive, was tested against the isolation of *Ps. tumefaciens* from which the bacteriophage was isolated, *Ps. pruni*, *Ps. beticola* (Smith, Brown, Town.) Potebnia, *Ps. citri* Hasse,

Ps. vesicatoria Doidge, *Ps. phaseoli* E. F. S., *B. carotovorus*, and *B. atrosepticus*. Cultures of these pathogenes had been inoculated previously into their respective hosts and their pathogenicity proved.

After making the required dilution of the lytic principle in 10 cc. of neutral broth, two drops of a 24-hour-broth culture of the organism to be tested were added to each tube. The results of this trial after four days growth are given in table 6.

TABLE 6.—*Effect of lytic principle V upon Pseudomonas tumefaciens and seven other plant pathogenes*[a]

Organism	Growth response at lytic filtrate dilutions								Check
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
Ps. tumefaciens	0	0	0	0	0	1–	1–	1–	4
Ps. pruni	2	2	2	2	2	2	2	2	2
Ps. beticola	3	3	3	3	3	4	4	4	4
Ps. citri	2	2	2	2	2	2	2	2	2
Ps. vesicatoria	2	2	2	2	2	2	2	3	3
Ps. phaseoli	2	2	2	2	2	2	2	2	2
B. atrosepticus	3	3	3	3	3	3	3	3	3
B. carotovorus	4	4	4	4	4	4	4	4	4

[a] Growth is indicated as follows:

0 = No growth of the organism, broth clear.
1– = Very faint haze but less growth than 1.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth with pellicle formation.

From table 6 it is seen that lytic principle V affected only the culture of *Ps. tumefaciens*, the other organisms growing as abundantly in the presence of the bacteriophage as in the untreated check cultures. At dilutions of 10^{-7} to 10^{-9} there was only a perceptible clouding of the cultures of *Ps. tumefaciens* with a few clumps of agglutinated bacteria, showing that the lytic principle exerted an inhibitory effect upon the organism although lysis was not complete.

Similar tests were also made with the same organisms, using lytic filtrate V after 16 passages through the Berkefeld W filter. In these trials complete lysis of *Ps. tumefaciens* took place at all dilutions 10^{-1} to 10^{-9}, inclusive, while the other plant pathogenes were not affected.

TABLE 7.—*Specific action of lytic principle V in different dilutions against Psudomonas tumefaciens isolated from different hosts*[a]

Number of culture	Source	Growth response at lytic filtrate dilutions						Check
		1–20	1–200	1–2,000	1–20,000	1–200,000	1–2,000,000	
1110	Apple	4	4	4	4	4	4	4
1162	Rose	4	4	4	4	4	4	4
R	Raspberry	0	0	0	0	0	1–	4
1212	Geranium	2	2	3	3	3	3	4
1215	Apple	4	4	2	3	4	4	3
1216	Apple	2	3	2	3	3	3	3
1217	Peach	3	3	3	4	4	4	4
1218	Apple	3	3	3	4	4	4	3
1219	Peach	4	4	4	4	4	4	4
1220	Walnut	3	3	3	2	2	2	3
1221	Apple	4	4	4	4	4	4	4
1223	Rose	2	2	2	2	2	2	2
1224	Weeping willow	4	4	4	4	4	4	4
1225	Western Sand cherry	4	4	4	4	4	4	4
1226	Raspberry	4	4	4	4	4	4	4
1227	Apple	4	4	4	4	4	4	4
1228	Incense cedar	4	4	4	4	4	4	4

[a] Growth is indicated as follows:

0 = No growth of the organism, broth clear.
1– = Doubtful growth.
1 = Very slight growth, very faint turbidity of broth.
2 = Slight growth, faint turbidity of broth.
3 = Moderate growth, slight turbidity of broth.
4 = Abundant growth, with pellicle formation.

Having shown that lytic principle V was univalent in its action when tested against seven other bacterial plant pathogenes, further trials were made using 15 isolations of the crown-gall organism from various hosts or from the same host at different times.

In this experiment lytic principle V was used after seven, eight, and nine successive passages through a Berkefeld W filter. A summarized record of the experiment at the end of four days is given in table 7.

The only culture which showed complete inhibition of growth was that of strain R (raspberry) from which lytic principle V was isolated. Culture 1226, a strain of the pathogene also isolated from a crown gall on a different raspberry plant, was not affected by the lytic principle. This failure of the lytic principle to affect culture 1226 indicates the specificity of the principle for the strain of the pathogene from which the bacteriophage was isolated. Further, the lability of the crown-gall organism is shown by the failure of the lytic principle to cause lysis of culture 1224. This latter culture was isolated from a six-months-old gall on weeping willow induced by inoculation with culture R, the strain from which lytic principle V was isolated. Such reaction indicates that the host may exert some influence upon the strain of the organism to change its reaction to the lytic principle.

LONGEVITY OF THE LYTIC AGENT IN CULTURE

Having shown that lytic filtrates III and V at dilutions of 10^{-21} prevented growth of *Ps. tumefaciens,* the question arose as to how long the filtrates would remain active without further addition of young cultures of the crown-gall bacteria and subsequent filtrations. Portions of filtrates III and V after the nineteenth passage through the Berkefeld filters were set aside on November 1. On January 20, dilutions of the filtrates in a series of 10^{-1} to 10^{-10} were made and one drop of a 24-hour broth culture of *Ps. tumefaciens* added to each tube. There was abundant growth in all of the tubes containing filtrate III, but no evident growth in filtrate V at the dilutions 10^{-3} and below. After two additions of young cultures of the crown-gall organism and subsequent filtrations, the potency of lytic principles III and V was again tested in a similar manner. At the dilution 10^{-5} no growth was evident four days after the addition of one drop of a 24-hour broth culture of *Ps. tumefaciens* to 5 cc. of the lytic filtrate culture. In subsequent trials made February 16 and March 3, there was only a very slight inhibition of growth of *Ps. tumefaciens* in both the stock filtrates. In these trials, the clouding of the culture was less than in the check broth tubes, and, in addition, there was a noticeable clumping of the bacteria 48 hours after adding one drop of the bacterial suspension to the filtrate. There was also a slight agglutination of the crown-gall bacteria at this time

in 1–10 dilutions of the filtrates. Growth at this dilution was almost as abundant as in the check tubes with the exception that no pellicle was formed. From these trials it appears that the bacteriophage does lose its potency upon prolonged standing in culture (about two and one-half months).

SUMMARY

A bacteriophage has been isolated from nonviable cultures of *Pseudomonas tumefaciens* (raspberry isolation) in sterilized and nonsterilized soil, from a virulent culture of the same isolation of the organism in broth, and from a crown gall on sugar beet. In high concentrations the lytic principle from all of these sources has caused complete lysis of *Ps. tumefaciens* in broth cultures.

Cultures were made by adding 1 cc. of a 24-hour broth culture of *Ps. tumefaciens* to 1, 2, 3, 4, and 5 cc., respectively, of lytic filtrate III after 14 filtrations. No infection resulted on young tomato plants from inoculations of the five cultures after being subjected to the action of the lytic agent nine hours or longer.

Injections of the lytic filtrate of known potency into young tomato plants failed to prevent infection when the pathogene was inoculated into the plant by means of the puncture originally made by the hypodermic needle.

The potency of the lytic agent was enhanced by additions of young broth cultures of *Ps. tumefaciens* to the stock filtrate along with successive filtrations through Berkefeld W filters. After 19 passages there was no evidence of growth of *Ps. tumefaciens* when added to dilutions 10^{-20} and 10^{-21}, respectively, of filtrates III and V. At higher dilutions growth was inhibited for 72 to 120 hours after inoculation but later became abundant.

The rough type of *Ps. tumefaciens* was recovered in poured plates from broth cultures of the lytic filtrate at dilutions of 10^{-3} to 10^{-5}, which showed no evidence of growth seven days after inoculation. Subcultures of the rough-type colonies were inoculated into other broth dilutions of the filtrate. From these cultures, showing moderate turbidity after seven days, *Ps. tumefaciens*, having the smooth-type colony, was plated out.

The potency of the lytic filtrate is greatly reduced by heating for ten minutes at 80° C. and completely inactivated at 85° C. for the same length of time. The lytic action of the bacteriophage is inhibited by streaking on bile agar.

The lytic agent when tried against *Ps. pruni, Ps. beticola, Ps. citri, Ps. vesicatoria, Ps. phaseoli, B. atrosepticus, B. carotovorus,* and *Ps. tumefaciens* inhibited growth of the crown-gall organism alone.

In trials using 17 strains of *Ps. tumefaciens*, the lytic agent affected only the culture of the organism from which it was isolated.

The lytic agent V tried against cultures of *Ps. tumefaciens*, R and 1226, isolated at different times from separate raspberry galls, affected only the former culture from which the bacteriophage was isolated. Culture 1224, a reisolation of culture R after passage through weeping willow, was not affected by lytic filtrate V.

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THE EFFECTS OF POTASH AND PHOSPHORUS ON TIP BURN AND MILDEW OF CABBAGE

CHARLES CHUPP

Fully ten years ago the writer was called into Monroe County, New York, to diagnose a certain trouble on cabbage. The plants in a third of a field had nearly every leaf showing dead, brown or black margins, with the lower leaves fairly well spotted by Alternaria. In the most pronounced cases whole plants were somewhat dwarfed and loose of head. The only difference in treatment to which the affected cabbage was subjected was a rather heavy side dressing with nitrate of soda. At that time the question arose as to whether the nitrate caused the plants to be more susceptible to Alternaria, which in turn might indirectly produce the tip burn effect.

Since then a similar injury was observed in many Danish Ballhead cabbage fields, but only occasionally on the early and kraut varieties. It soon was determined to be worse some years than others, and on some farms than others. It seemed more prevalent when fall-plowed soil became too hard in the spring to be made friable by cultivating tools or when the ground was puddled in the spring when it was plowed. There seemed to be no correlation between the amounts of the trouble on high ground and low ground, although at times it was noticeable that the sloping land gave rise to more tip burn than did the level stretches in the same field. Neither did acidity nor alkalinity seem to play an important part in the production of the malady.

All of these observations, together with the fact that some of the best growers maintained that they could not grow Danish cabbage after turning under a heavy alfalfa or clover crop, led to the conclusian that the tip burn was the direct result of too large an application of nitrogen and that possibly the addition of a certain amount of superphosphate (acid phosphate) would counteract the supposed injurious effects of the nitrogen. It was some time before this supposition could be tested under field conditions, but, as the tip burn became more of a problem, and when the growers insisted that help was necessary if successful growing of the late crop was to be continued, experiments were undertaken in 1927. These dealt only with superphosphate and nitrate of soda. Not even a check without fertilizer could be included, as Mr. E. N. Reed of Cortland County, the grower who kindly had consented to cooperate, had treated all of his field with the superphosphate. Therefore, the only thing undertaken was to apply to part of the field a double amount of the superphosphate, then divide the area into plots on some of which no nitrate was added, on others the same

amount that Mr. Reed usually applied for cabbage, and on still others twice the amount of nitrate. One plot had three times as much nitrate of soda as was the usual application on this farm. All the other plots were replicated seven times. Each plot consisted of four rows of 20 plants each.

The arrangement of the plots was faulty, since all the double amounts of superphosphate were on one half of the experimental field and those with single applications on the other half. Fortunately, however, the regular part of the field was quite uniform in its amount of tip burn. The results, therefore, have some value in determining the effects of sodium nitrate and superphosphate on the prevalence of the disease.

TABLE 1.—*Effects of phosphorus and nitrogen on tip burn in 1927. Reed farm*

Pounds of fertilizer applied per acre		Percentage of tip burn
16 per cent superphosphate	Nitrate of soda	
1250	166	27.78 ± 1.104
1250	332	17.22 ± 4.924
1250	None	27.22 ± 6.389
625	166	12.78 ± 3.408
625	332	12.44 ± 4.285
625 (One plot)	498	17.00 ——
625	None	9.44 ± 0.613

The results were unexpected, for the percentage of tip burn was increased rather than lessened where the amount of superphosphate was doubled. It also is true that where the amount of sodium nitrate was tripled the increased tip burn was very noticeable, but, where it was merely doubled, the amount of tip burn was not nearly so great as it was in the doubled superphosphate plots.

The question then arose in regard to the importance of each of these two fertilizer ingredients in the production of tip burn and just what effect potash would have alone or in combination with either one of them. Previous work by Newhall[1] suggested that potash or nitrogen may increase the severity of tip burn in lettuce. Would it do the same in the case of cabbage? It was remembered, however, that the tip burn in lettuce begins on the younger, more tender leaves, and spreads outward to the older ones, while, in cabbage, the opposite is true, and that only when the trouble is severe are the inner leaves injured enough to reduce the size of the plant and resulting head. Might not, therefore, the reaction of cabbage to potash differ from that of lettuce?

[1] Newhall, A. G. Studies on the tip burn disease of lettuce. Phytopath. **15**: 58. 1925.

FIG. 1. Tip burn on a cabbage leaf.

In 1928 rather detailed experiments were made on the Reed farm, Cortland County, and on the George Maier farm, Monroe County, to test more fully the effects of the various fertilizer elements on the disease. Incidently, the Maier farm was one where tip burn had become so troublesome that it was being considered unprofitable to grow cabbage for storage. The yield was reduced and the harvested cabbage had to be either trimmed too closely for good keeping in storage or left with tip burned leaves from which secondary rots spread to various depths in the head.

TABLE 2.—*Arrangement of the plots on the Maier farm, 1928*

N–PP–K	P–N	P–K	P–P	P	N–PP–K	P–N	P–K	P–P	P
N–P–KK	K–N	K–K	P–K	K	N–P–KK	K–N	K–K	P–K	K
NN–P–K	N–N	K–N	P–N	N	NN–P–K	N–N	K–N	P–N	N
N–P–K	N	K	P	O	N–P–K	N	K	P	O

P = Acid phosphate, N = Nitrate of soda, K = Potash, O = Check.

The arrangement on the Reed farm was similar, but had no complete fertilizer included. The plots in each of the experiments consisted of four rows and twenty plants in each row. There were not so many checks nor so large a number of replications as was desired because of limited space.

The soil on the Reed farm was a Lordstown slit loam and on the Maier farm, Ontario fine sandy loam. On both places the soil was in excellent condition, having in the past years received heavy legume cover crops, some barnyard manure, and a definite amount of commercial fertilizer. The cabbage crop in both cases was very good, except for some tip burn, especially on the Maier farm.

TABLE 3.—*Effects of fertilizer on tip burn in 1928*

Treatment	Percentage of tip burn	
	Reed farm	Maier farm
N–PP–K	———	34.04 ± 11.357
PP	46.83 ± 8.792	13.63 ± 1.810
P	38.32 ± 9.621	26.62 ± 9.172
PN	16.78 ± 2.876	26.88 ± 10.269
NN	1.05 ± 0.355	18.42 ± 2.456
N	2.44 ± 0.644	17.54 ± 2.507
PK	7.79 ± 1.922	7.73 ± 2.798
NN–P–K	———	7.16 ± 0.161
N–P–K	———	5.87 ± 1.604
KN	1.25 ± 0.326	6.96 ± 1.189
K	4.83 ± 1.805	9.44 ± 2.04
KK	1.56 ± 0.913	9.34 ± 3.241
N–P–KK	———	3.70 ± 0.608
O (Check)	9.98 ± 3.545	15.72 ± 6.178

PP, NN, KK = Double amounts of these materials.

The applications in all cases, throughout the three years, were made after the plants were set into the field, excepting the first year, when the superphosphate was applied before the planting. The material for each individual plot was weighed separately and strewn over the soil by hand as uniformly as possible. A cultivator was then used to cover the fertilizer. On the Reed farm 333 pounds of nitrate of soda, 625 pounds 16 per cent superphosphate, and 200 pounds of muriate of potash (approximately 5–8–8 formula) were applied on the acre basis. In the case of the Maier farm the number of pounds was 80, 220, and 40, respectively (approximately 3–10–6 formula).

Counts of tip burn were made late in September. It was difficult to obtain accurately the amount of the disease present. At first an attempt

was made to count each tip burned leaf. This was very laborious and, besides, did not give a true representation, for some of the most seriously affected plants had fewer leaves because of the disease. Therefore, it was finally decided to divide the degree of infection into three classes: Mild, medium, and severe. Then, in getting the percentage, the number under severe was multiplied arbitrarily by three and that under medium by two. The sum of these was added to the figure under mild. Thus the figures in the tables would represent or be equivalent to the given percentage of mild tip burn. At least, the method gave uniformity in measuring the amount of injury and seemed better than merely counting the affected plants. The data were collected without consulting the copied plan of the experiment, so that the judgment would not be biased in making the counts. In addition, other workers were asked to aid in the checking, to insure greater accuracy in the results.

As might be supposed by working with anything as variable in its composition as the soil, there was much variation in the percentages obtained. This was true particularly because, on the Reed farm, there was only a medium amount of rainfall, while there was more of it on the Maier farm. In consequence, the nitrogen seemed to reduce injury in the drier field and had little or no effect in the wetter one. These results with nitrogen were all the more striking, since, the following year, the condition in the two counties was just reversed. Still, nitrogen apparently reduced the amount of tip burn in the dry soil and had little effect where there was more rainfall. It is for this reason that the combined figures for the three seasons are too variable in the case of nitrogen to say definitely what the results will be for any given year.

Notwithstanding the variability in results from nitrate of soda, the effects from superphosphate and muriate of potash were surprisingly uniform whether the soil was relatively wet or dry. Superphosphate, when used without potash, always increased the amount of tip burn. Potash always decreased it. For instance, single applications of superphosphate in 1928 gave 38 and 27 per cent of tip burn, while the plots without fertilizer gave 10 and 16 per cent and the single potash plots gave 5 and 9 per cent. The double superphosphate plots on the Maier farm gave a much smaller percentage increase than was expected, but PP plots gave 47 and 14 per cent, respectively, on the two farms, in contrast to the 2 and 9 per cent of the KK plots.

In 1929 the experimental plots were discontinued on the Reed farm, because the method of fertilizing seemingly furnished so nearly a properly balanced ration for the soil that tip burn was not serious enough to warrant the drawing of conclusions. Mr. Claude Rorapaugh of Cortland, who rented a farm quite depleted in plant food, kindly consented to act as a

cooperator. He permitted the use of an area large enough to supply sixty-four plots, of four rows each and thirty-seven plants in the row. The remainder of his field had an application of 600 pounds an acre of 16 per cent superphosphate and 300 pounds of a 10–10–10 mixture. The soil was a Chenango stony silt-loam type, which grew a large yield of cabbage. The experiment was continued on the Maier farm in Monroe County, although in another field. There also were sixty-four plots of four rows each with twelve plants to the row. A portion of the remainder of the field had 450 pounds of 5–10–5 fertilizer and another portion 200 pounds of 0–12–18. The Rorapaugh farm had plenty of rain, but on the Maier farm there was almost none after the cabbage was set out.

TABLE 4.—*Arrangement of plots on Maier and Rorapaugh farms, 1929*

P	K	N	O	KN	N	PN	NN	KN	PK	K	KK	P	PN	PK	PP
PN	KN	NN	N	PK	P	PP	PN	N	P	O	K	K	KN	KK	PK
PK	KK	KN	K	K	O	P	N	PN	PP	P	PK	N	NN	PK	PN
PP	PK	PN	P	KK	K	PK	KN	NN	PN	NO	KN	O	N	K	P

On both farms the single applications of the fertilizing elements were the same as represented in a thousand pounds per acre of 5–10–5. Where the

TABLE 5.—*Effects of fertilizer on the amount of tip burn on the Maier and Rorapaugh farms in 1929*

Treatment	Percentage of tip burn	
	Maier farm	Rorapaugh farm
PP	37.95 ± 4.263	5.70 ± 0.826
P	30.82 ± 5.601	7.21 ± 1.451
(5–10–5) (450 lbs.)	26.00 ± 3.028	———
PN	15.74 ± 2.305	8.86 ± 2.610
O (Check)	11.17 ± 3.032	1.04 ± 0.375
PK	10.92 ± 2.350	1.36 ± 0.477
K	6.29 ± 1.702	0.17 ± 0.108
N	4.16 ± 0.952	1.65 ± 0.604
KK	5.15 ± 1.504	0.00
KN	2.54 ± 0.556	0.21 ± 0.132
NN	1.62 ± 0.287	0.37 ± 0.215
(10–10–10) (+ 600 lbs. P)	———	2.50 ± 0.859
(0–12–18) (200 lbs.)	0.20 ± 0.224	———

TABLE 6.—*Summary of experiments on the effects of potash and phosphorus on the tip burn of cabbage*

Treatment of plots	Order of tip-burn severity in the different experiments													
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
PP	2	1	1				1							
N–PP–K	1													
PPN	1													
PN	1	1	1	1										
P		3	1			1								
PPNN			1											
O (Check)			1	2		1	1				2			
NN				1				1						
10–10–10 + 600 lbs. P.				1										
N					2			2						
PNN					1									
PK					2					1				
5–10–5						2	2			1				
K								1	3		1			
KK									1	2		1		
KN											1	1		
NN–P–K													1	
0–12–18														1
N–P–K														
N–P–KK														

letters are doubled a double portion of this element was applied. The material was strewn as uniformly as possible by hand after the plants were set into the field and covered with the cultivator. The results in 1929 were substantially the same as those obtained in 1928. The effects of the nitrogen again were variable, but, in general, seemed to reduce the amount of tip burn. The superphosphate increased the amount of tip burn, and potash decreased it, as is shown by table 5.

In none of the three years while the experiments were being conducted did the chosen fields show much tip burn. For this reason some of the probable errors are too large to make the figures significant. But, wherever tip burn was at all abundant it was easy to recognize a superphosphate plot from that which received potash. This is brought out fairly clearly when all the data are summarized in one table.

The difference may be represented also by averaging the percentages of the five experiments.

TABLE 7.—*Tabulated summary of the results of three years' experiments on the effects of potash and phosphorus on tip burn of cabbage*

Treatment of plots	No. of experiments	Percentage of tip burn
PP	5	26.266 ± 4.525
P	5	22.481 ± 3.039
PN	5	16.210 ± 1.810
NN	4	5.365 ± 1.774
KK	4	4.01 ± 1.214
K	4	5.18 ± 1.126
KN	4	2.74 ± 0.867
O (Check)	4	9.48 ± 1.794

In 1928 Quanjer[2] mentions a case where cabbage grown in experimental plots deficient in potash had much tip burn. Cauliflower, also, showed harmful effects under the same conditions, but the symptoms were of a different nature. In the plots where the potash was supplied both crops were healthy, thereby showing that the lack of potash was the cause of the trouble. In a still more recent publication Schoevers[3] illustrated currant leaves that were dwarfed and tip-burned where potash was lacking and showed the healthy branches from plots where this mineral was supplied. It seemed that the form potassium sulphate was more desirable than potassium

[2] Quanjer, H. M. De invloed van kaligebrek op de vat baarheid van bloemkool voor *Peronospora parasitica*. Tijdschr. Plantenziekten 34: 254–256. 1928.

[3] Schoevers, T. A. C. Een proef met zwavelzure kali tegen "Randjesziekte" bij roode bessen. Tijdschr. Plantenziekten 35: 231–233. 1929.

chloride. Many other cases in literature could be cited where some form or ingredient of fertilizer was capable of changing the immunity or the susceptibility of host plants to various types of diseases. Some of these cases have been proved beyond doubt, while many others are little more than conjectures based on very meager experimentation.

The data given in this discussion may not seem complete enough to justify much generalizing, but the results of the experiments, together with many observations, have persuaded the writer that the farmers have at hand a means of controlling their cabbage tip burn. On a number of farms a fertilizer high in potash, as 5–8–7, was used successfully in the control of the trouble and also induced excellent yields. The formula 4–12–4, used on some farms, increased tip burn wherever the disease was present. In one community, where the growers still clung to the old-time formula of 2–8–10, it was very noticeable that the late cabbage was not affected by tip burn. This statement is not meant as a recommendation of such a formula but is given merely to show that fertilizer with a high potash analysis sometimes is desirable.

No attempt was made to determine the reason why lack of potash or an increase of phosphorus causes tip burn; but in a talk with a plant physiologist it was suggested that potassium is in some way connected with the respiration processes and is found most abundantly in the meristematic tissue. In a plant depleted of this mineral, the potassium may be withdrawn from the older tissues and localized in the meristematic areas. On the other hand, an increase in phosphorus, an element necessary to cell division, might tend to increase the growing area and thus hasten the potassium withdrawal from the leaves. Because of the consequent incomplete respiration, this would be followed by the presence of toxic substances that might cause the dying of the leaf tissue.

No plot yields are given because, where single elements were used, the yield necessarily was reduced. Furthermore, no attempt was made to get the combination of fertilizer that would produce the highest yield. That is a subject for the agronomist and not for the pathologist.

Quanjer,[4] in the article previously quoted, discussed the effect of potash on the presence of downy mildew of cabbage and cauliflower (*Peronospora parasitica* (Pers.) de Bary). He came to the conclusion from his observations that downy mildew was much more severe in the plots lacking potash. He mentioned also work that had been done on *Plasmopara viticola* and *Bremia lactucae* in which quite similar results were obtained. He is inclined to believe that all downy mildews might react in the same manner.

[4] *Loc. cit.*

The fertilized plots for tip burn in 1929 had a fairly large amount of downy mildew on the leaves and, therefore, afforded an opportunity for obtaining information regarding the influence on this disease. The data obtained in the two counties are given below.

TABLE 8.—*Effects of fertilizer on the downy mildew of cabbage*

Treatment	Average number of mildewed leaves in the plots	
	Rorapaugh farm 74 plants counted per plot	Maier farm 48 plants per plot
KK	72.345 ± 2.704	14.325 ± 2.533
K	71.446 ± 5.389	12.525 ± 1.449
PK	56.031 ± 7.150	11.700 ± 3.260
KN	53.449 ± 5.527	16.037 ± 2.314
NN	43.360 ± 6.961	11.725 ± 1.487
N	40.714 ± 2.989	19.950 ± 2.717
PN	31.939 ± 4.580	19.875 ± 4.333
O	28.347 ± 1.910	11.175 ± 3.032
P	25.647 ± 6.113	5.237 ± 0.722
PP	19.910 ± 2.561	1.825 ± 0.580
0–12–18	.....	22.600 ± 2.239
5–10–5	.....	22.000 ± 1.780
(10–10–10) (+600 P)	14.292 ± 1.275	.....

It was less difficult to record the amount of mildew than of the tip burn. The number of spots on a leaf was correlated very closely with the number of affected leaves per plant. Therefore the record was taken by counting the number of affected leaves in each plot.

The information at hand is insufficient to permit drawing conclusions, especially since the figures for the Maier farm are so variable. But the results of the one year seem to be diametrically opposed to the observations of Quanjer. It would be interesting to follow this with at least two more years of experimentation to prove more fully the effect of the different fertilizer ingredients on the downy mildew. Since this is not possible at the present time, these observations and figures are given with the hope that some one else will study the subject more fully.

When recording the data the potash plots were evident because there was little tip burn and much downy mildew. The superphosphate plots could be recognized because of just the opposite results. The nitrogen also seemed to increase the mildew. This was true both on the Maier farm where rainfall was almost entirely lacking and on the Rorapaugh farm where the rainfall was abundant. On the latter farm potash very evidently increased mildew, while on both farms phosphorus decreased it. A table

giving the odds in comparing some of the plots having potash with those having superphosphate will give something regarding their relative importance.

TABLE 9.—*Odds as obtained by the "Student's" Table in comparing some of the mildew plots*

Treatment of plots	Plots with which compared	Odds showing relative significance of difference	
		Rorapaugh farm	Maier farm
KK	PP	1249 to 1	16 to 1
K	P	2499 to 1	293 to 1
PK	PN	28 to 1	1 to 4
KN	PN	97 to 1	1 to 2
NN	O	3 to 1	1 to 2
K	N	2499 to 1	1 to 3
O	PP	4 to 1	3 to 1
KK	O	4999 to 1	1 to 1

There was almost three times as much mildew on the Rorapaugh farm as on the Maier farm which lacked the summer rains. This aids in making the odds insignificant on the latter place. But on both farms there is evidence to show a significant difference between the K and P plots and even the KK and PP ones, although the results from the double applications were not so consistent as were those from the single amounts of potash and phosphorus.

As was stated above, no conclusions may be drawn from the two experiments. But, even if potash were found to increase downy mildew, the fact would be of little economic importance; for, under New York State conditions, mildew never is serious enough in the field to cause any reduction in yield. Therefore, the recommendation that a fertilizer formula with a larger percentage of potash be employed in combating tip burn can still be followed with no fear of increasing another trouble. The only place where downy mildew might ever be of consequence is in the seed bed, so that if the data on the Rorapaugh farm later prove correct the seed bed could be protected from mildew by a heavy application of superphosphate and a correspondingly light one of potash.

SUMMARY

Tip burn of cabbage is fairly general and sometimes severe on Danish Ballhead cabbage grown in New York State. The trouble rarely is found on kraut and early varieties. Heavy applications of superphosphate (acid

phosphate) increased the amount of tip burn, especially where nitrogen and potash were lacking or were present in small amounts.

Nitrate of soda had little effect for or against the production of tip burn. It seemed to increase the disease when the applications were heavy.

Muriate of potash reduced the amount of tip burn enough to recommend it as a practical control measure. The fertilizer ratio 1–2–2, as represented by the formula 5–8–7 or similar ones, is approximately the correct one on the farms where the experiments were conducted.

Downy mildew of cabbage was present very prominently on one farm where the tip-burn experiments were conducted, and a small amount on the second farm.

Preliminary experiments indicate that potash and nitrogen may increase the amount of downy mildew of cabbage and that phosphorus seems to decrease it. The one year's results do not permit the drawing of conclusions.

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RELATION OF ENVIRONMENTAL FACTORS TO GROWTH AND PATHOGENICITY OF PYTHIUM ISOLATED FROM ROOTS OF SUGAR CANE

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The disease of sugar cane usually referred to as root rot and growth failure is of great economic importance in most sugar-producing countries. The disease retards the growth of the cane, following a deterioration of the roots. Investigators have attempted for many years to determine the cause of this disease, and, from time to time, various organisms or soil conditions have been stated to be important factors. The prevailing opinion as has been brought out by Edgerton, Tims, and Mills (5), is that a number of factors are involved.

One of the factors generally recognized to be important in the root-rot problem is the root decay caused by species of Pythium or Pythium-like fungi. Carpenter (2), Bourne (1), and Edgerton and his coworkers (4, 5) have all shown that certain species of Pythium attack the roots and produce a soft, flabby rot of the root tips. The percentage of roots thus affected under field conditions is frequently very high. At the same time, the investigations in Louisiana (5) have shown that the species of Pythium capable of rotting cane roots are universally present in fields of healthy cane as well as in those suffering from root rot. From this, it seems possible that the severity of Pythium injury depends more or less on environmental conditions.

To obtain some information on the importance of various environmental factors in the root-rot problem a series of experiments have been conducted to determine the influence of moisture, temperature and hydrogen-ion concentration on the growth of Pythiums in culture and on the development of Pythium root rot of corn.

In the infection experiments, corn was used instead of sugar cane. Earlier investigations at the Louisiana Agricultural Experiment Station had shown the behavior of the parasitic Pythiums almost the same on both. Sugar cane is propagated vegetatively, and, because the buds usually germinate very unevenly and the young plants grow slowly during the winter months, it cannot be so readily employed in infection experiments as corn, nor does it yield such reliable results.

TEMPERATURE STUDIES OF PYTHIUM IN CULTURE

The effect of temperature on the rate of growth of sugar cane Pythiums was obtained with eight different cultures. Four of these (931, 1432, A,

and B) were parasitic forms isolated from cane roots in Louisiana. These produced the lobed, enlarged hyphae characteristic of the species known to be the most important form found on cane. A fifth culture (1285), which came from Hawaii, was obtained from cane roots affected with the Lahaina disease. This culture also produced enlarged hyphae. Two other cultures (1436 and C), also isolated from cane roots in Louisiana, were only slightly parasitic. The eighth culture (D), although isolated from decaying cane roots, was of a nonparasitic species.

To obtain the effect of temperature on these organisms transfers were made from the margins of rapidly growing cultures to petri dishes containing Difco dextrose agar. The plates were placed in constant-temperature incubators which had a temperature range of 15° to 36° C. The increase in diameter of each colony was measured at 12-hour intervals and the average growth in three plates obtained. The results are given in table 1.

TABLE 1.—*The influence of temperature on the rate of growth of sugar-cane Pythiums on dextrose agar*

Temperature, degrees C.	Culture number or designation							
	931	1432	A	B	1285	C	1436	D
	Diameter increase of colonies in 12 hours (cm.)							
15	1.3	1.1	1.0	0.9	1.1	0.9	0.9	0.7
20	2.1	2.0	1.8	1.7	1.9	1.9	2.1	0.8
25	2.5	2.6	2.6	2.5	2.6	2.5	3.2	1.0
30	2.8	2.9	2.9	2.9	2.9	3.2	4.0	1.1
36	1.4	1.7	1.7	1.4	2.0	2.5	3.2	0.5

As shown in the table, the growth of all the parasitic cultures was nearly the same. The rate of growth increased with the temperature up to 30° C. and fell off markedly at 36° C. Thirty degrees was also the most favorable temperature for the mildly parasitic forms, but these made a fairly good growth at 36° C. The last two cultures seemed to be somewhat different, 1436 growing considerably faster than C at all except the lowest temperature. The nonparasitic form grew slowly at all temperatures.

Although the differences in the rate of growth of the parasitic cultures were slight, the results indicated that at lower temperatures 931 grew faster than 1432, while at higher temperatures the reverse was true. Because of this, these two cultures were studied at narrow temperature ranges. From this study it was found that, at 25° C., the rate of growth was approximately the same for both. At temperatures below 25°, 931 grew somewhat faster

than 1432, while at temperatures above 25° it grew more slowly. These results are of interest because 931 had been growing in culture for three years and 1432 only one year. In this time 931 had become decidedly less parasitic than it was at first.

RELATION OF H-ION CONCENTRATION TO GROWTH

Early in the investigations on the root-rot problem it was observed that parasitic Pythiums were rarely obtained when diseased roots were plated on acidified media but were readily obtained on nonacidified media. It was thought desirable therefore to obtain more information concerning the influence of H-ion concentration on the growth of Pythium.

A number of nutrient solutions were used in preliminary trials. The one judged most suitable consisted of 20 grams of dextrose, 3 grams of beef extract, and 5 grams of peptone made up to 1000 cc. with distilled water.

The usual technique for this type of work was employed. The beef extract, peptone, and sugar were obtained from the Digestive Ferments Company. Pyrex glassware and water distilled over block tin were used. The H-ion determinations were made colorimetrically with LaMotte indicators. Because sugars react with acids and alkalies when heated under pressure, these were sterilized separately. The nutrient solution was made up to 600 cc. instead of a liter. Flasks of 250 cc. capacity were used, 60 cc. of the concentrated solution being placed in each flask. Sufficient distilled water was then added so that, when the required amount of tenth-normal acid or alkali was added after sterilization, the total volume in each flask was 100 cc. These flasks were inoculated and the amount of growth was determined at weekly intervals by collecting the mycelium on a weighed filter paper dried to constant weight in an oven at 100° C. The paper and mycelium were weighed after these had been similarly dried.

Twenty-four flasks of medium were prepared for each H-ion concentration to be tested. Eighteen flasks were inoculated with Pythium 1432 by transferring a small portion of the medium and mycelium from the margin of a three-day-old dextrose-agar culture to each flask. Six flasks were kept as controls. It had been planned to determine the pH and amount of fungous growth in three inoculated and one control flask each week for a period of six weeks, but contaminations limited the experiment to five weeks. The results of the tests are given in table 2.

The results in the table indicate that a neutral or slightly alkaline medium is most favorable for growth of Pythium. The fungus did not grow in solutions of initial pH 4.3 or 4.6. Growth was retarded at first in those flasks having an initial pH of 5.3, but by the end of three weeks it was as great as in solutions of a more favorable H-ion concentration. Best

TABLE 2.—*Relation of hydrogen-ion concentration to growth of sugar-cane Pythiums on acid and alkaline media for different lengths of time from date of inoculation*

N/10 HCl or NaOH in 100 cc.	pH after 72 hours	*1 week*		Weight mycelium in mg.	*2 weeks*		Weight mycelium in mg.	*3 weeks*		Weight mycelium in mg.	*4 weeks*		Weight mycelium in mg.	*5 weeks*		Weight mycelium in mg.
		pH			pH			pH			pH			pH		
		Check	Pythium		Check	Pythium		Check	Pythium		Check	Pythium		Check	Pythium	
7.5 cc. HCl	4.3	4.3	4.2	0	4.2	4.2	0	4.2	4.3	0	4.2	4.2	0	4.3	4.3	0
5.0 cc. HCl	4.6	4.6	4.6	0	4.6	4.6	0	4.6	4.6	0	4.6	4.6	0	4.6	4.7	0
2.5 cc. HCl	5.3	5.3	5.6	54	5.2	5.8	338	5.3	6.0	598	5.2	6.7	628	5.2	7.9	532
Control	6.0	6.0	6.0	160	6.0	6.0	484	6.0	6.2	571	6.0	6.9	581	5.8	8.2	500
10 cc. NaOH	8.3	8.2	6.6	238	8.3	6.2	518	8.2	6.6	603	8.2	7.8	533	8.1	8.2	487
20 cc. NaOH	9.2	8.7	7.8	90	8.8	6.4	428	8.7	6.0	518	8.7	7.6	517	8.6	8.3	445
30 cc. NaOH	9.6[a]	9.0	8.6	31	9.1	7.2	283	9.0	6.4	404	9.0	6.5	543	8.8	7.9	486
40 cc. NaOH	9.6[a]	9.6[a]	9.6[a]	0	9.2	9.2	0	9.2	9.2	0	9.2	9.2	0	9.1	9.1	0

[a] Higher than 9.6 not determined for lack of proper indicators.

growth was obtained in the solution having an initial pH of 8.3, but growth in the solution to which no acid or alkali had been added was only slightly slower. The most alkaline media were beyond the range of the indicators, but growth was greatly retarded or stopped at a pH greater than 9.6.

The metabolic products of fungous growth changed the pH of the media towards 6.0 regardless of the initial H-ion concentration. This tendency to change the pH towards 6.0 continued as long as the mycelial felt increased in weight. After maximum growth had been reached, the solutions uniformly became alkaline, as shown in the tests made after four and five weeks.

THE INFLUENCE OF TEMPERATURE ON INFECTION

In order to determine the effect of temperature on the rotting of roots by Pythium, corn was grown in No. 2 metal cans in temperature-controlled incubators. The cans were filled with soil and then sterilized in the autoclave for two hours at 15 pounds pressure. Before planting, the soil was inoculated with pure cultures of Pythium, strains 931 and 1432. A single three-day-old petri-dish culture of the fungus on bean-pod agar served as inoculum for each can, and a similar amount of sterile bean-pod agar was placed in each control can. Five germinating seedlings of the variety Calhoun Red Cob were planted in each can. Three cans of each Pythium and the same number of checks were grown at each temperature. The soil was maintained at 60 per cent of its water-holding capacity by weighing daily. The plants were allowed to grow and the height was obtained by measuring to the end of the longest leaf. The amount of rot present on the roots was also observed. The results of this test are given in table 3.

TABLE 3.—*Influence of temperature on growth of corn in Pythium-infested soil in temperature incubators*

Temperature degrees C.	Period of growth	Average length of tops in cm.			Percentage decrease	
		Check	Pythium 931	Pythium 1432	Pythium 931	Pythium 1432
35	5	24.2	25.8	22.2	6[a]	8
30	5	30.0	23.5	19.0	22	37
26	5	26.8	15.4	12.0	43	55
23	5	17.4	12.2	10.8	30	38
20	10	30.0	19.0	19.6	37	35
15	20	19.5	10.1	9.3	48	52

[a] = Increase.

Both strains of Pythium were severely parasitic at the intermediate and lower temperatures, but 931, which had been in culture three years, ap-

peared to be less parasitic than 1432, which had been in culture only one year. A temperature of 35° C. was too high for the best growth of corn, but at this temperature there was very little rotting of the roots. At 30° C. the corn grew best, although there was considerable rotting. As the temperature was lowered, Pythium injury to the roots became more severe, and at 15° C. even the main roots were rotted off nearly to the seed. These results agree closely with those of Johann et al. (6), who found that *Pythium arrhenomanes* Drechs., which causes a root rot of corn in Wisconsin and is very similar to the cane Pythium (3), was more pathogenic at low than at high temperatures and in wet than in dry soils.

Another form of Pythium injury was noted at the low temperatures. In a number of cases there was a rot of the mesocotyl and the stem above the seed, and the Pythium mycelium was found in the decayed areas. This type of injury was not found on corn grown at temperatures above 23° C. An occasional plant was found partly rotted at 23° C., but at 20 and 15° C., this type of rotting was quite prevalent.

INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON PYTHIUM INFECTION

To determine the effect of soil moisture on Pythium infection on corn roots, a series of tests was run in the greenhouse in the spring of 1928. Cans of No. 2 size were filled with a good mixed soil and, after sterilization in the autoclave at 15 pounds pressure for two hours, each was inoculated with strain 931, a three-day-old petri-dish culture of a parasitic sugar-cane Pythium. Each can was then planted with surface-sterilized corn kernels of the variety Calhoun Red Cob. Water was then added so that the moisture content of the soil varied from 20 per cent to 100 per cent of the maximum water-holding capacity of the soil. Quadruplications of each variant were used. In order to determine the effect of temperature variations also, the test was run every month from March to June. The soil temperatures in the greenhouse during the test periods were as follows:

	Minimum	Maximum	Mean
	°F.	°F.	°F.
March	56	67	60
April	51	72	63
May	64	79	71
June	75	88	82

The germination of the seed and the height of the plants were used as criteria of Pythium injury. The results of these tests are given in tables 4 and 5.

TABLE 4.—*Influence of soil moisture and temperature on the growth of corn in Pythium-infested soil*

Percentage of water-holding capacity of the soil	Length of tops in cm.							
	March 1–11		April 2–21		May 2–21		June 8–19	
	Check	Pythium	Check	Pythium	Check	Pythium	Check	Pythium
20	5.6	4.3	3.0	3.0	8.5	7.5	4.6	3.3
30	12.2	8.1	10.9	11.9	18.7	16.9	8.3	6.3
40	14.9	9.8	16.5	12.7	26.4	22.3	16.2	13.1
50	17.2	10.8	20.9	12.1	34.0	22.2	23.1	19.9
60	17.8	10.3	25.0	11.6	38.4	19.7	26.7	18.5
70	18.3	8.1	22.6	9.1	36.4	16.3	28.3	18.4
80	18.9	10.0	23.4	8.5	38.8	11.5	29.6	17.2
90	14.5	0.0	20.6	0.0	0.0	0.0	28.6	15.4
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 5.—*Influence of soil moisture and temperature on the germination of corn in Pythium-infested soil*

Percentage of water-holding capacity of the soil	Percentage of germination							
	March 1–11		April 2–21		May 2–21		June 8-12	
	Check	Pythium	Check	Pythium	Check	Pythium	Check	Pythium
20	12.5	7.5	2.5	10.0	77.5	75.0	12.5	10.0
30	95.0	92.5	87.5	77.5	97.5	90.0	27.5	45.0
40	100.0	95.0	97.5	90.0	90.0	95.0	92.5	80.0
50	97.5	80.0	97.5	85.0	92.5	90.0	97.5	95.0
60	87.5	55.0	80.0	57.5	85.0	77.5	90.0	95.0
70	77.5	37.5	62.5	32.5	80.0	45.0	97.5	95.0
80	70.0	15.0	30.0	20.0	12.5	17.5	85.0	72.5
90	35.0	0.0	12.5	0.0	0.0	0.0	67.5	40.5
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The data in table 4 show that reduction in growth due to Pythium was most pronounced during the cooler months and in the wetter soils. Soil maintained at 20 and 30 per cent of its water-holding capacity was too dry for good growth of corn, but in this dry soil there was little Pythium injury. As the soil moisture was increased, the difference between the corn growing in the inoculated and in the noninoculated soils became greater, but the size of the plants in the Pythium soil increased with the increase in moisture content until the 50 per-cent series was reached. In Pythium soil kept wetter than 50 per cent of its water-holding capacity, the corn plants decreased in size with increase in soil moisture; whereas, in the noninoculated

soil, the plants increased in size with the soil moisture until the 80 per-cent series was reached. Injury due to Pythium was also more pronounced during the cool than during the warm months. In March, April, and May, the corn growing in the Pythium-infested soil kept at 50 per cent of its water-holding capacity was only about 60 per cent as tall as the check, while, in June, it was 85 per cent. In figures 1 and 2 are shown plants from the March and May series.

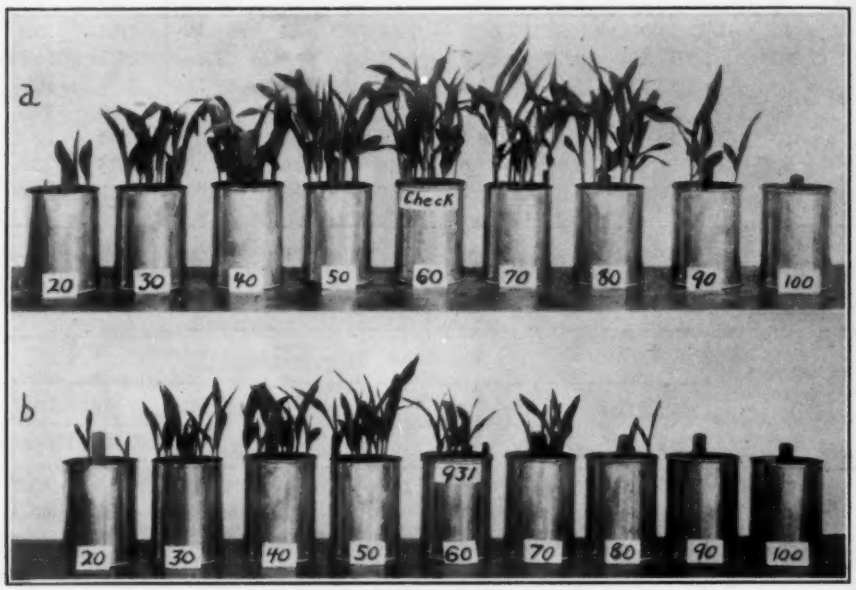

FIG. 1. Relation of soil moisture to Pythium injury. (a) Corn plants grown in sterile soil with the moisture ranging from 20 per cent to 100 per cent of the water-holding capacity of the soil. (b) Similar to the above, except the soil was reinoculated with Pythium. Growth from March 1 to March 10, 1928.

The germination results are quite regular except at the extreme moisture variations where the soil was too dry or too wet. The inoculation of the soil with Pythium did not materially affect the germination of corn in soil kept at 30 or 40 per cent of its water-holding capacity. In the series conducted during the cool months of March and April there was a noticeable reduction in germination at 50 per cent in the Pythium-infested soil; in the May series this reduction was evident at 60 per cent; while, in June, the 80 per-cent Pythium-infested soil was the first to show a significant reduction in germination. These tests indicate that both the growth and germination of corn in Pythium-infested soil are closely correlated with soil moisture and temperature.

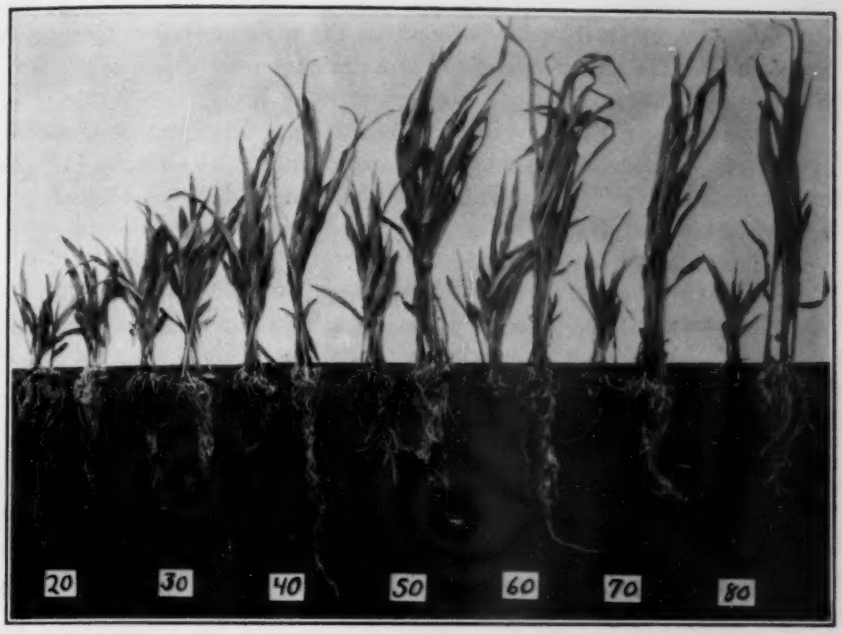

FIG. 2. Relation of soil moisture to Pythium injury. Corn plants grown in sterile soil and in sterile soil reinoculated with a pure culture of Pythium. Of each group of two plants the one on the left was from the inoculated soil. Moisture content of soil varied from 20 per cent to 80 per cent of the water-holding capacity of the soil. Growth from May 2 to May 21, 1928.

DISCUSSION

These studies were undertaken in order to obtain additional information regarding the environmental factors which influence the severity of the decay of cane roots caused by species of Pythium. The results obtained have given some interesting information in regard to the effect of temperature, soil moisture, and soil acidity.

It is known that root rot of sugar cane is more severe in poorly drained fields and in seasons of excessive rainfall (4) and exceptionally low temperature in January, February, and March. This is explained to a certain extent by the results given in this paper. Although the growth rate of Pythium increases with the temperature up to 30° C., the effect of the fungus on growing plants is more severe at lower temperatures. Only young terminal portions of the roots are subject to attack and it is possible that the cool weather holds the roots in a susceptible condition longer than warm weather.

The results obtained in the soil-moisture studies agree closely with conditions in the field. The effect of the fungus was severe only in soils with a

water content greater than 50 per cent of the moisture-holding capacity. This emphasizes the importance of the best drainage possible, especially during winter and spring, when the cane grows very slowly.

The hydrogen-ion studies show that the Pythium fungi can grow readily in any of the Louisiana soils used for cane culture. The results indicate that the fungi grow well from pH 5.6 to 9.2, and most of the cane soils are in the neighborhood of the neutral mark.

SUMMARY

1. The growth rate of strongly parasitic, mildly parasitic, and nonparasitic cultures of Pythium, isolated from the roots of sugar cane, increased with the temperature up to 30° C. The rate of growth of the strongly parasitic cultures fell off sharply at 36° C.

2. A parasitic Pythium grew well in solutions of pH 5.3 to 9.2. It did not grow at pH 4.6.

3. Pythium injury to germination and growth of corn decreased with rise in temperature. At 35° C. Pythium did not injure corn. At 30° C. there was an appreciable amount of injury and, as the temperature was lowered, injury became more severe.

4. Pythium injury to germination and growth of corn increased with the water content of the soil but was less severe in wet soils in warm than in cold weather.

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THE CHERRY LEAF-SPOT FUNGUS, MYCOSPHAERELLA CERASELLA ADERH., ITS MORPHOLOGY AND LIFE HISTORY

W. A. JENKINS[1]

Toward the end of summer, cultivated cherries in North Carolina become prematurely defoliated by the conidial form of a fungus commonly referred to as *Cercospora cerasella* Sacc. This organism appears to be of rather common occurrence on the several species of cherries but is of little economic importance anywhere. It has been reported to be present on *Prunus avium* L. in Georgia and Alabama, on *P. demissa* D. Dietr. and *P. virginiana* L. in Kansas, and on *P. cerasus* L., both sweet and sour varieties, in Alabama, Mississippi, and Florida (2). Saccardo (6) records its occurrence on cherries in France and Italy and Aderhold (1), in Germany.

This leaf-spot disease can be distinguished readily from such diseases as shot hole, rust, and mildew, which are more or less generally prevalent throughout the State. Its lesions are at first pin-point-like, circular, yellow spots present on either leaf surface. As they enlarge to their mature diameter of a millimeter or more, the central portion becomes reddish brown with a border of lighter color. The necrotic tissues gradually become dry and brittle and when the lesions are abundant they may coalesce to form large, irregular, dead areas. In the case of severe infections rather serious defoliation results. The reserve food stored for the succeeding season is in consequence diminished. Either or both leaf surfaces of mature lesions are covered with dark fascicles of conidiophores and conidia.

Apparently the only published account of the disease and of the life history of the pathogene is that of Aderhold (1) in 1900. Beginning in 1894, he observed the disease throughout several seasons and noted that during winter an ascigerous stage appeared on fallen leaves near old Cercospora lesions. He isolated the organism from ascospores and obtained in these cultures conidia of a Cercospora together with structures which he interpreted to be perithecial initials. He also observed that fallen diseased leaves, which remained out of doors during winter and were then brought into the laboratory during April and May and placed in moist chambers, developed conidiophores and conidia of the Cercospora type. On the basis of these findings he concluded that the ascigerous stage, which he designated *Mycosphaerella cerasella* n. sp., is the perfect stage of *Cercospora cerasella* Sacc.

The present study, which extends over the past two years, deals with the development of the fungus in leaves, in both natural and artificial

[1] Acknowledgment is made of the counsel and help of F. A. Wolf, under whose direction this study was conducted.

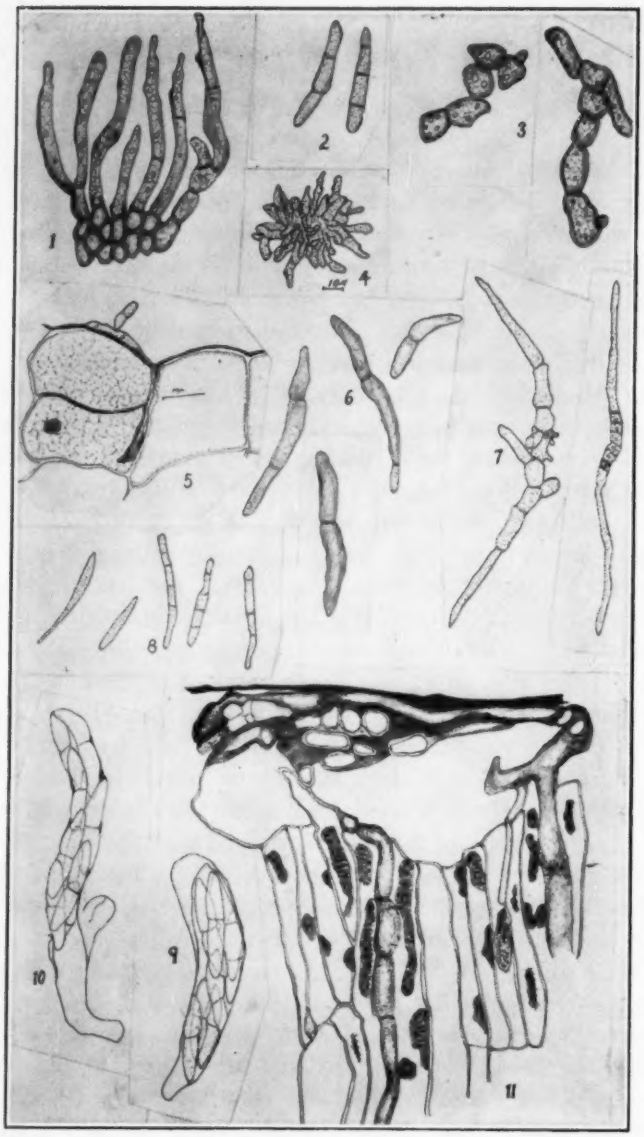

FIG. 1. 1. Conidiophores arising from a stromatic base; 2. Conidia developed in culture; 3. Chlamydospore-like cells of the type commonly present in cultures; 4. Conidiophore fascicle in surface view; 5. Penetration of germ tube from conidium, in transverse section of leaf; 6. Germination of ascospores of *Mycosphaerella cerasella* Aderh.; 7. Germination of conidia; 8. Conidia from leaf lesions; 9. Mature ascus; 10. Ascus which has elongated preparatory to ascospore discharge; 11. Relationship of pathogene and suscept shown in transverse section of lesion. 1 and 11 drawn to scale shown near figure 2, 13; 2, 3, 5, 6 and 8–10 to scale near 5; 4 and 7 to scale near 4.

inoculations, and in artificial cultures, with special emphasis on the morphology and genetic relation of the several stages. It therefore supplements the previous study by Aderhold (1).

Infected leaves were collected at frequent intervals throughout the year. They were then fixed in Bouin's fluid, appropriately sectioned, and stained with Haidenhain's iron alum haematoxylin.

The germ tubes from conidia sown on the surface of living leaves were found entering the leaves by direct penetration (Fig. 1, 5). It would appear that they could enter also through the stomata which occur on the dorsal leaf surface only. The mycelium is at first confined to the intercelluar spaces, but upon death of the cells it enters them (Fig. 1, 11). There is no evidence that the pathogene is able to kill cells in advance or actual extension of the mycelium.

CONIDIAL STAGE

By the time that well-defined lesions are evident the conidial stromata have begun to develop subcuticularly or within substomatal cavities. As the result of continued growth compact masses of stromatic tissue are developed, which serve as the bases of the conidiophores. (Fig. 1, 1.) These conidiophores extend outward and project from the leaf surface either through fissures in the cuticle or through the stomatal orifices. Each conidiophore fascicle consists of a small group of spreading elements (Fig. 1, 4), hyaline at first and brown at maturity, from the tips of which the conidia are abjointed. As the conidia mature they, too, gradually become brown. After a conidium is abstricted and falls away, leaving a scar, the conidiophore continues to grow beyond this scar. By the repetition of of this process the conidiophore becomes geniculate and flexuose. The conidia are obclavate, bent, pale brown, septate, guttulate, and measure 40–60 by 3–4 μ (Fig. 1, 8).

SPERMOGONIA

Spermogonia are formed on both leaf surfaces of fallen leaves throughout the fall and early winter. They originate either within old conidial stromata or within separate stromata that develop after the death of the leaves. Mature spermogonia were first noted in sections of material fixed during the latter part of October and spermatial production ceased by the latter part of December.

In studying the course of spermogonial development use was made of sections of infected leaves and of spermogonia produced in artificial culture. They arise meristogenously (Fig. 2, 16 and 17) by the branching of a hypha and the fusion of these branches. Then, by a rapid division

of cells, a spherical stromatic mass is formed (Fig. 2, 18). The cells at the center of this mass, which are to become the spermatium mother cells, become larger and stain deeper than those of the periphery. The nucleus of each mother cell then divides and 3–4 spermatia are formed endogenously (Fig. 2, 19). The spermatia are liberated through a sterigma-like process, figure 2, 19, after which the walls of the old mother cells disintegrate into a mucilaginous matrix which invests the spermatia. This process continues centrifugally so that a mature spermogonium (Fig. 2, 20) consists of a globose cavity, filled with spermatia held together in a mucilaginous matrix of degenerating mother-cell walls. Outside of this is a layer of fertile cells densely filled with protoplasm and then the spermogonium wall, itself, consisting of two or three cell layers of brown, thick-wall elements. Mature spermogonia measure 36–90 by 25–73 μ and spermatia 2.5–2.7 by 0.9–1.0 μ.

This method of spermatial formation appears to be quite similar to that described by Higgins for *Mycosphaerella bolleana* (4) on fig and *M. personata* (5) on grape. Whether or not it is of any phylogenetic significance is conjectural, especially since no Ascomycetes are known to give rise to conidia in this manner.

PERITHECIA

Perithecia form most abundantly on the surface of the leaf in contact with the soil. They begin their formation about the same time as do the spermogonial primordia but do not become sufficiently differentiated to be recognized as perithecial primordia until late November, about a month after mature spermogonia are first noted. At this time the developing stromata have differentiated into a peripheral portion with brown, thick-wall cells and a medullary portion with thin-wall cells, rich in protoplasm. In this central portion there is a coiled, coenocytic carpogonium which arises near the base of the stroma and projects, by means of a trichogyne, nearly or entirely through the cells at the apex of the stroma (Fig. 2, 12). Apparently, each stroma normally has a single carpogonium, although two perfectly formed ones were observed in one case.

As growth continues, more deep-staining cells form around the carpogonium. Apparently these cells function as a source of nourishment for the developing carpogonium and later, those which remain, as a reserve for the rapidly growing asci. Meanwhile the trichogyne degenerates, beginning at the tip, and the basal portion becomes the ascogonium. By December 12, ascogonia of the type shown in figure 2, 13, had formed. Various stages in this process of differentiation could be found in collections made during the period extending from late November to late December. Each ascogonium remains coenocytic but the nuclei become as-

sociated in pairs, presumably by nuclear migration. At least there is no evidence that one of the members of each pair of nuclei comes from an antheridium or any structure remotely resembling an antheridium as described in *Venturia inaequalis* (Cke.) Wint. (3). During the period which follows, the ascogonia enlarge and the nuclei multiply, apparently by conjugate division. Further, there is no evidence of fusion of these paired nuclei within the ascogonia. Instead, they remain associated with no actual fusion except within the young asci which may first appear several weeks later.

Various stages in the development of asci were observed in collections made in the period extending from the latter part of December to the middle of March. Asci arise as branches extending outward from the ascogonium. There is no evidence of crozier formation. Instead, each ascus is a separate branch originating directly from the ascogonium so that each ascogenous hypha is actually a young ascus (Fig. 2, 15). This is entirely like the condition described in *Mycosphaerella bolleana* (4).

Not all asci arise at the same time nor do they develop at the same rate and, consequently, do not mature at one and the same time. Within a single perithecium various stages in development of asci can be noted, ranging from very young ones to mature ones. It would therefore be anticipated that there would be an extended period of ascospore discharge from any individual perithecium. In fact, the period of spore expulsion has been found to extend from the middle of March throughout the entire summer and into early September. This sustained period can be accounted for in part, by the lack of uniformity in development of asci within individual perithecia. The further observation that, in March, some perithecia were in the same state of maturity as were others in the previous December may also account, in part, for this sustained period of ascospore discharge.

A pair of nuclei migrate from the ascogonium into each young ascus and there fuse, thus constituting the primary fusion nucleus. This is followed by three succesive nuclear divisions, so that the normal number of eight free nuclei is formed. The ascospores are next delimited uniseriately and at first each is continuous and uninucleate. Then follows a division of each ascospore nucleus and the septation of each ascospore into two unequal cells, the upper of which is the larger. Meanwhile, they have become oriented in distichate fashion. Mature ascospores range in size from 13–17 by 3–4 μ.

The asci are clavate-cylindrical with a thickened, slightly excentric papilla. They measure 40–60 by 8–10 μ (Fig. 1, 9). The walls are composed of two membranes the outer of which ruptures and is thrust aside as the inner membrane elongates to about twice its normal length preparatory to expulsion of the ascospores (Fig. 1, 10). By this mechanism the

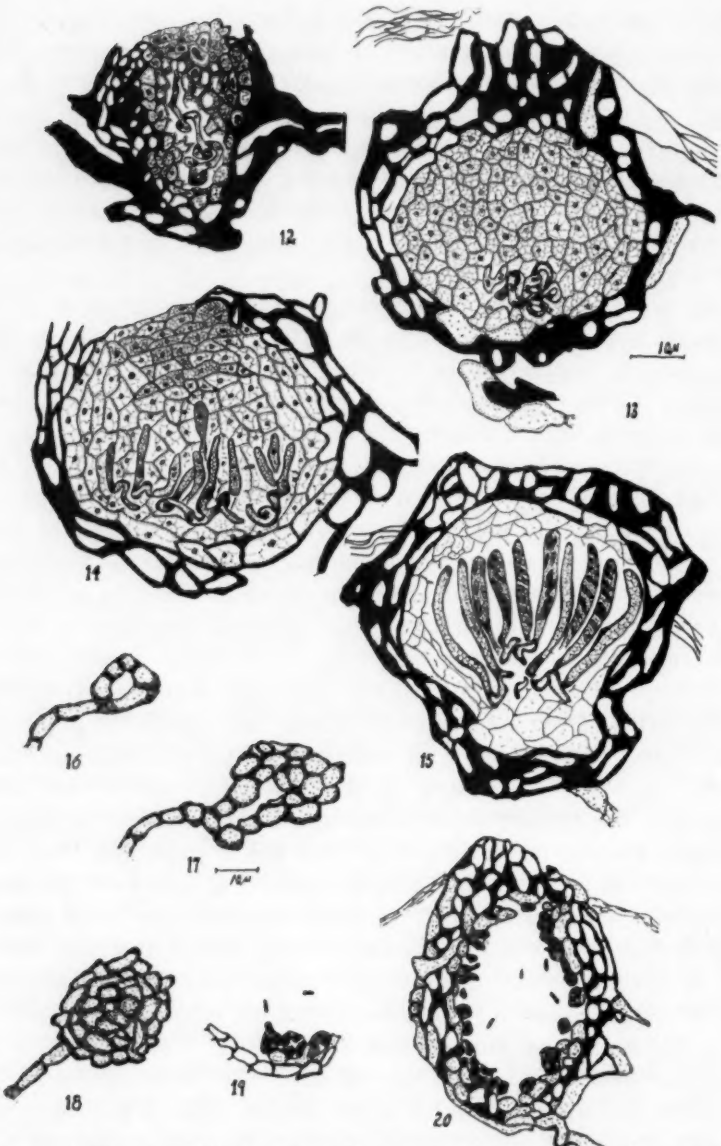

FIG. 2. 12. Young perithecial primordium showing coiled coenocytic carpogonium; 13. Later stage in perithecial formation showing ascogonium with paired nuclei; 14. Young asci arising from an ascogonium; 15. Perithecia with well-developed asci just prior to the formation of the ostiolum; 16, 17, and 18. Successive stages in spermogonial formation; 19. Portion of spermogonial wall showing spermatium mother cells and spermatia; 20. Nearly mature spermogonium in detail with only a few spermatia which normally fill the central cavity. 12–15, drawn to scale shown near 13; 16–20 to scale near 17.

asci extend into the ostiolum and discharge their spores to the exterior. They then collapse and thus afford opportunity for spore discharge by other asci within the same perithecium.

The stromata from which perithecia arise have in some cases been found to be the stromata which had previously borne conidiophores, in others, separate stromata, but they are always located wholly within the leaf tissues. As the perithecia approach maturity, during ascus formation, they either rupture the epidermis or distend it and appear, under the hand lens, as dense clusters of black projections. In section these show that each perithecium remains closed with no evidence of an ostiolum until the ascospores are essentially mature. Each perithecium is capped by a tissue of brown, thick-walled cells (Fig. 2, 15). Just beneath this cap and above the asci is a tissue of thin-walled, fragile cells similar to those which disintegrate as the asci enlarge. It would appear that pressure exerted by the developing asci must push apart the lateral walls of the perithecium and thus tend to form a pore or ostiolum at the top. At the same time the thin-walled tissue above the asci proliferates and, in so doing, forms a lining for the ostiolum. This type of development of ostiola resembles that recently described by Higgins (5) for *Mycosphaerella personata*.

GENETIC RELATIONSHIP OF THE SPORE FORMS

The genetic connection of conidia, spermogonia, and perithecia of the cherry leaf-spot fungus rests upon the following evidence:

(1) Spermogonial and perithecial primordia have been observed to arise, as has already been stated, in the bases of conidial stromata within fallen leaves. Moreover, a characteristic mycelium consisting of thick-walled, large, brown cells ramifies throughout the leaf tissues, extending from the conidiophore bases and also from spermogonia and perithecia and could readily be traced from any one of these structures to any other.

(2) Typical Cercospora conidia have been developed in artificial cultures made from ascospores of this leaf-spot organism. Curiously, when conidia or ascospores were planted on such media as potato agar, corn-meal agar, bean agar, dextrose agar, synthetic nutrient agar, or cherry-leaf-decoction agar, to each of which fragments of diseased leaves had been added as an additional source of nutriment, no fruiting was obtained, although the fungus made very satisfactory vegetative growth. If, however, tubes of these media, on which the organism had been growing for a time, were resterilized and reinoculated, conidia were produced (Fig. 1, 2), together with spermogonia bearing spermatia and stromatic bodies which were apparently perithecial primodia, when cultures made from ascospores were the source of inoculum. When the inoculum came from cultures

made from conidia the results were identical, except that conidia were not observed to have been developed. Neither asci nor ascospores, however, were ever noted in these stromatic bodies.

All efforts to germinate spermatia both from leaves and from culture failed. This failure accords with the observed behavior of spermatia of other Ascomycetes.

(3) Small cherry trees, the leaves of which showed no signs of infection, were set in the greenhouse in December and inoculated on April 4 with ascospores. Infection resulted and on April 25 mature lesions had developed which bore conidia of *Cercospora cerasella*. The ascospores used in these inoculations were obtained by placing perithecia-bearing leaves beneath inverted agar plates. The spores, on expulsion, lodged on the surface of the agar above. In inoculating, blocks of agar with adhering ascospores were cut out and placed in contact with the leaves. The leaves were than wrapped for 24 to 48 hours in oiled paper to conserve moisture. Inoculated leaves, only, became infected.

An unidentified species of Mycosphaerella with somewhat smaller ascospores than the pathogene was found to be rather commonly associated with it on overwintered leaves. This unidentified organism was isolated in pure culture and compared with *M. cerasella* from which it is distinct in all vegetative characters. No conidia developed in these cultures. Moreover, no infections were secured following inoculation by the same method successfully employed with the pathogene.

(4) The development of conidial, spermogonial, and ascigerous stages by the cherry leaf-spot fungus, in sequence in the same lesion, is in accord with an increasing body of knowledge of the course of development in the life cycles of Ascomycetes.

These observations and results, when regarded as a unit, justify the conclusion that the three stages, herein described, belong to one and the same fungus, *Mycosphaerella cerasella*. Since, in this study, the complete life cycle of the cherry leaf-spot fungus is determined for the first time, a brief description of it is included.

MYCOSPHAERELLA CERASELLA ADERH.
SYN. CERCOSPORA CERASELLA SACC.

Ascigerous stage: Perithecia densely aggregated, amphigenous, innate but erumpent at maturity, globose, black, ostiolum only slightly papillate, 60–120 μ in diameter; asci clustered, bluntly clavate-cylindrical, aparaphysate, thickened by an excentric papilla at the apex, eight-spore, 40–60 by 8–10 μ; ascospores imperfectly biseriate, unequally bicellular, the upper cell somewhat larger, slightly curved, hyaline, 13–17 by 3–4 μ.

On fallen decaying leaves of *Prunus* spp., maturing during the period extending from March to September.

Spermogonial stage: Spermogonia densely scattered in and near lesions produced by the conidial stage, globular to flask-shape, black, embedded in the leaf tissue at first, later erumpent, ostiolate, 36–93 by 25–72 μ; spermatia rod-shape, 2.5–2.7 by 0.9–1.0 μ, arising endogenously, usually in fours within spermatiferous cells, and liberated by sterigma-like projections, hyaline.

On recently fallen leaves, maturing throughout the period extending from late October throughout December.

Conidial stage: Lesions circular to irregular, often confluent, varying in size, pale brown to dark brown; conidiophores amphigenous, arising from a stroma, spreading in a lax fascicle, geniculate, flexuose, brown at maturity, 30–40 μ long; conidia cylindrical to clavate, straight or curved, ochraceous to pale brown, guttulate, septate, 40–60 by 3–4 μ.

On living leaves of *Prunus* spp. in late summer.

SUMMARY

This report deals with a leaf-spot disease of cherries, caused by *Mycosphaerella cerasella*, which has three spore forms in its life cycle. Partial proof of the genetic connection of its conidial stage and its asciger-ous stage has been submitted by Aderhold in 1900. The evidence of this connection, herein submitted, is sufficient to regard it as conclusive proof. In addition there is a spermogonial stage which appears during autumn.

The morphology and course of development of each of the three spore forms are described.

The pathogene has been isolated in pure culture in which conidia and spermogonia are produced. Pathogenicity has been established by inocula-tion experiments.

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AN OFFSHOOT AND LEAFSTALK DISEASE OF DATE PALMS DUE TO DIPLODIA[1]

HOWARD S. FAWCETT[2]

In January, 1928, the attention of the writer was called to this disease on offshoots of date palms, *Phoenix dactylifera* L., by members of the staff of the United States Department of Agriculture Experiment Date Garden, Indio, California, who have given helpful cooperation throughout this investigation.

The disease had been observed by the writer on leafstalks in one date-palm garden the preceding year. It has since been found in a number of gardens throughout the Coachella Valley. One of the date growers reports having observed the offshoot form of the disease ever since date growing was established in that section. The disease appears to be most common on the Deglet Noor variety.

SYMPTOMS

In severe cases the disease results in death of offshoots either while they are still attached to the mother palm or after they have been detached and planted out. The disease sometimes also causes a premature death of leaves in older palms.

In offshoots the disease manifests itself in two distinct types; one, in which the outside leaves die first and the younger shoots and bud remain alive for some time; the other, in which the dying-back of the center of offshoot or bud precedes the death of the older leaves. It appears from the investigation and observations thus far carried on that the manner and place of infection largely determine which of these two types will occur. The infection appears to take place either at the base of the offshoot, near the place where it joins the mother palm, and probably prevents sufficient water from getting to the young leaves, or the older infection takes place in the outside leaves of the offshoot first and spreads later to the newer leaves and to the heart.

In the leaves of older palms the ventral midportion of the stalks is the part most commonly affected, showing yellowish brown streaks running upward 6 inches to 3 or 4 feet from the base (Fig. 1, A). The disease may spread laterally from one leaf base to others in close proximity. Frequently, these streaks extend upward on one of the lateral angles of the

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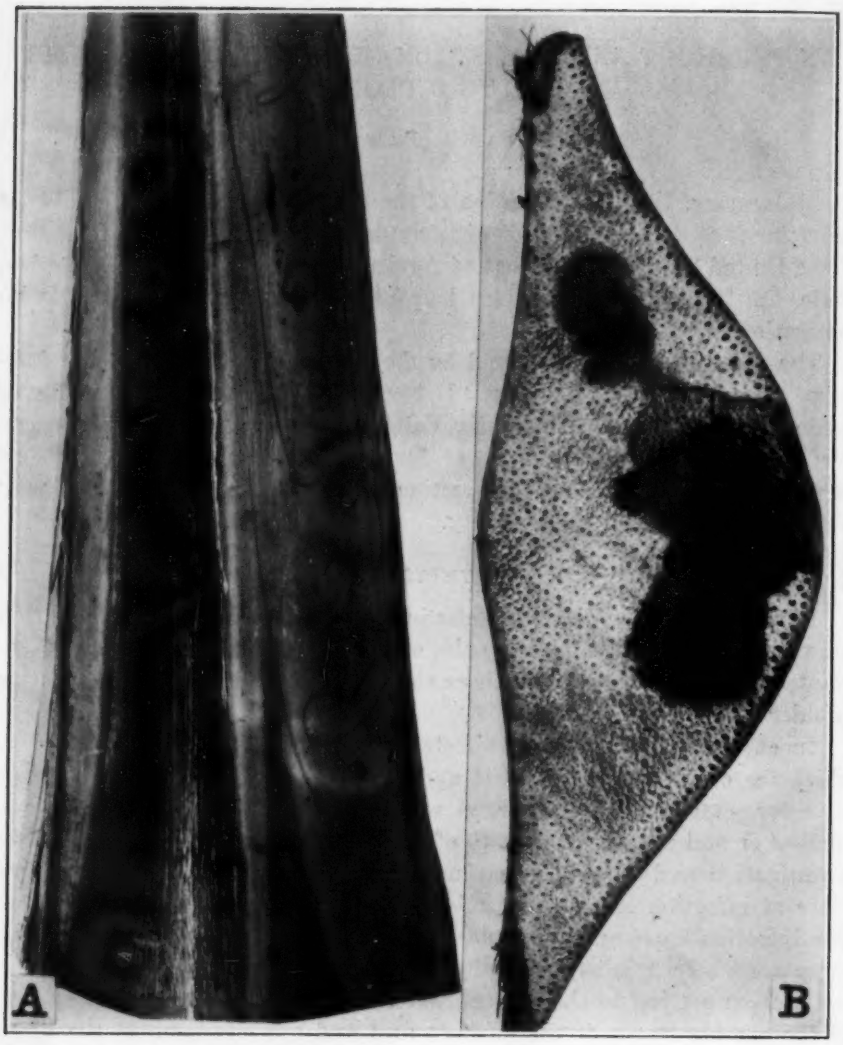

FIG. 1. A. Leafstalk of *Phoenix dactylifera* invaded by Diplodia showing discolored streak extending up from base at the midventral portion (about one-half natural size.) B. Cross section showing region of dark brown tissue in the interior of a leafstalk invaded by Diplodia. (About natural size.)

leafstalk. These streaks may sometimes extend upward long distances, while the upper portion of the leaf is still normally green and apparently unaffected. The color and size of these streaks vary according to the age of the leaves. On a leaf still green these streaks may be 3 to 4 inches wide at the base, narrowing rapidly upward to a width of half an inch or less.

The outside color near the base may be pecan brown to terra cotta. On a cross section (Fig. 1, B) the tissue may appear chestnut brown to raw umber at the center and cinnamon buff on the edges where the tissue has been more recently involved. In a large leaf these brown streaks have been observed to extend as much as 4½ feet from the base and beyond this point to extend 2½ feet farther, as a yellow streak.

ISOLATION AND INOCULATION EXPERIMENTS

Early in 1927 isolations of a Diplodia were made from the interior tissue of streaks in a leaf base and inoculations were carried out on leaf bases of a healthy palm in the same orchard in March, 1927. While infection was seen to be taking place after several months, its progress seemed to be slow and the inoculated plant was not carefully watched. Two years after the inoculation was made, not only were the infected leaves dead, but a near-by offshoot on the same palm was found badly infected. The angles of the petioles of three other leaves nearest those inoculated showed characteristic streaks extending upward from the base. The disease in this palm had all the appearances of having definitely progressed from the inoculated leaf bases. From these streaks in the leaf bases, as well as from the diseased offshoot, Diplodia was again isolated.

In January, 1928, Diplodia was again isolated from the discolored tissue of a dying offshoot in another date garden. This Diplodia culture was inoculated into the leaf bases of healthy palms in this date garden in March, 1928. In three months the largest lesion was 5 inches long and 1½ inches wide and, in seven months, it was 7 inches long and 1¾ inches wide (Fig. 2, B) and had spread inwardly almost entirely through the interior tissue of the leafstalk. This leaf was removed and Diplodia was again isolated in pure cultures from the advancing margins of the lesion. A number of other lesions, somewhat smaller in size, were produced at the same time.

A similar set of inoculation experiments was carried on in a third date garden from which Diplodia had been isolated from the leaf bases of older palms, and these gave similar results. The same fungus has been isolated from two other date orchards, making five in all. These experiments indicate that this Diplodia is the causal agent in the disease.

CONTRIBUTING FACTORS

Observations indicate that, although wounds made in pruning contribute to infection leading to the development of the disease (Fig. 2, A), such wounds are not always necessary to infection; especially, in case of offshoots. For example, in one Deglet Noor garden where the disease is doing much harm to offshoots, no pruning of leaves on either the mother trees or their

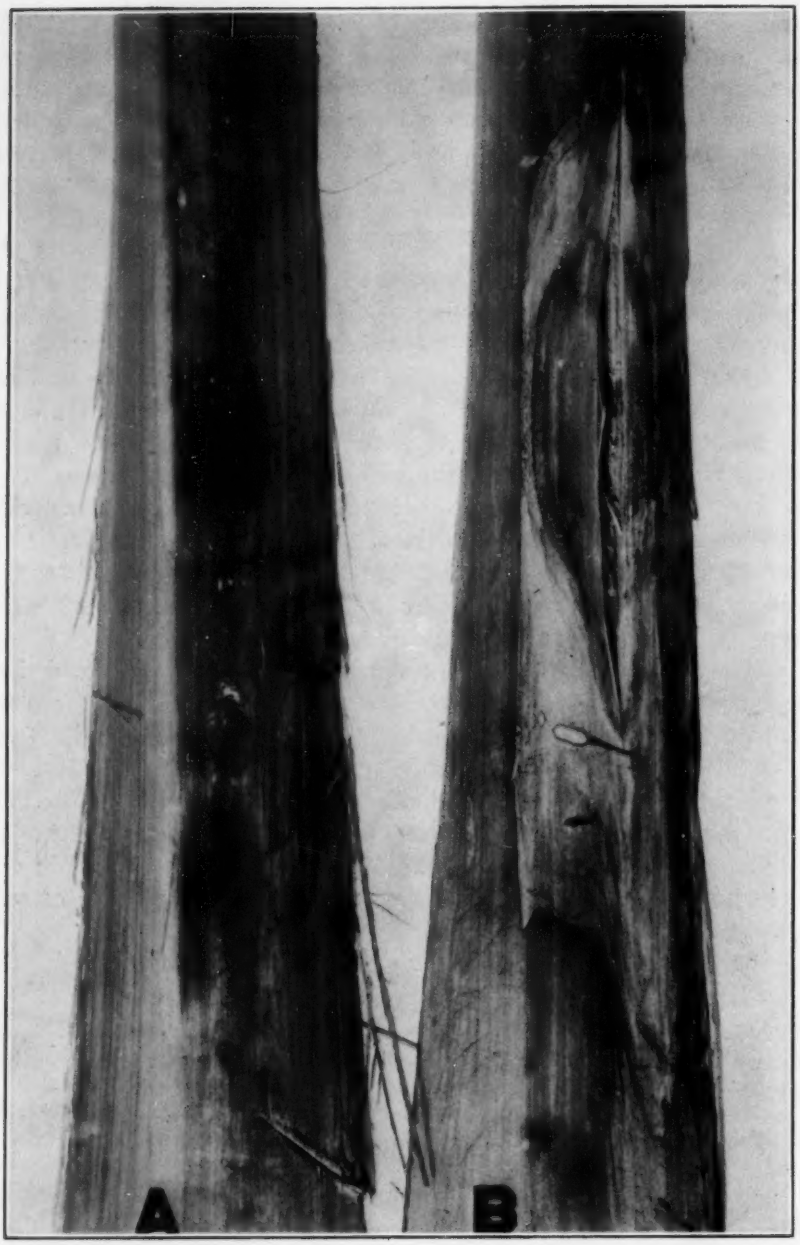

FIG. 2. Lesions on leafstalks of *Phoenix dactylifera* resulting from Diplodia infection. A. By means of an inoculation at cut occurring accidentally in pruning. B. By means of an inoculation from culture of the fungus purposely placed in a small cut near middle of present crack (crack has been elongated during development of lesion).

offshoots has ever been done except when cuts were made in removing offshoots from the mother palms for planting. These are growing in good soil and have been growing very rapidly as the result of being well fertilized and well irrigated. Many growers have assumed that dying of offshoots under such conditions was due to "crowding off" by the very rapid growth of the palms. It is possible that in some cases this rapid growth may cause strains on the union between the offshoot and mother palm, causing cracks in which infection could more readily take place. This point has not been sufficiently investigated. The type of the disease which begins first in the outer leaves either of the offshoots or of the older palms appears to be aided by wounds. Another condition that may contribute to the disease is severe defects in irrigation where main roots may be accidentally allowed to dry out and die back. Such accidental defects in irrigation practice had been reported in some of the gardens where the disease was most prevalent on the leaves of older palms. A number of other organisms also have been found which are probably secondary, but some of them may aid in increasing the severity of the disease. The principal fungi sometimes found associated with the Diplodia are *Penicillium roseum*, *Aspergillus niger* v. Tiegh., and species of Fusarium and Rhizopus.

CAUSAL FUNGUS

The Diplodia found in the diseased tissue is similar in size of spores to *Diplodia natalensis* Evans. It seems probable, however, that it is a different strain from the *Diplodia natalensis* that produces stem-end rot and a type of gummosis of Citrus in Florida. The fungus has not been found attacking Citrus fruits nor the bark of Citrus trees growing close to date palms in the date gardens of the Coachella Valley.

The spores are at first unicellular and hyaline like those of the form genus Macrophoma, but later, under certain conditions, they become dark and bicellular and measure 22 to 24 μ by 10 to 12 μ. The light-color spores are of about the same size. The pycnidia have not been found on the leaf bases of the older palms while they are still alive and attached to the tree under conditions in Coachella Valley, but are common on dead leaf parts at certain times. They are formed also on inside protected dead leaves of dying offshoots; and, if the stained interior tissue of the lesions on leaf bases is placed in a moist chamber, pycnidia are formed on the surfaces.

SUGGESTIONS FOR CONTROL

While results have not yet been obtained from control experiments, certain suggestions may be made. Observations strongly indicate that infection readily enters the wounds made by tools in pruning the leaves

(Fig. 2, A). It would, therefore, appear to be a proper precaution to disinfect all tools and cut places in operations where cuts have to be made. The cut surfaces made in removing offshoots from the mother plant should be disinfected and as much as possible of the dead and functionless tissue removed, disinfected, or burned. One of the date growers has reported beneficial results by such practice in planting out new offshoots in which he has removed any discolored tissue at the base and disinfected the cuts with a mixture containing 2 per cent of commercial Formalin[3] and 98 per cent water. It is also suggested that the tools be disinfected with the Formalin or some other good fungicide. As a further means of prevention, it is suggested that the palms be sprayed with an ammoniacal solution of copper carbonate. This will probably penetrate the crevices between the leaf bases better than Bordeaux mixture, since it is a clearer liquid than the latter. It is also likely to spread more readily on the waxy surface because of its more alkaline nature. Five ounces of copper carbonate, 3 pints of ammonia (26° Baumé) to 50 gallons of water may be used. The ammonia should first be diluted with 1 gallon of water. The copper carbonate is made into a paste with water and slowly added to the dilute ammonia, after which water is added to make 50 gallons.

[3] Commercial Formalin is a mixture containing 40 per cent of formaldehyde gas.

INSECTS AS POSSIBLE CARRIERS OF THE CITRUS-SCAB FUNGUS[1]

ANNA E. JENKINS

The importance of insects in the transmission of plant disease is already recognized in the literature, as shown by Rand and Pierce's (8) compilation. These authors employ the term *mechanical internal transmission* as applying to "that type of insect transmission" in which the "infective principle remains viable after passage through the alimentary tract but undergoes no appreciable multiplication or development within the insect body." In this connection it is (8, p. 205) explained that the "organisms that pass through the insect's body are in a large number of instances also carried externally. . . ." Also, it is stated (8, p. 207) that "as many as seven species of fungi causing diseases in plants have been shown capable of passing through the intestinal tract in a viable condition." Beetles, as well as flies and ants, are included among the insect carriers. None of the fungi named are Sphaceloma species nor are any of them related to this myriangioid group. Furthermore, among the beetles no Mycetaeid has been reported as a transmitting agent.

The present account contains more or less limited data relating principally to the mechanical internal transmission by insects of the Sphaceloma causing scab of Citrus, which fungus species (*Sphaceloma fawcettii* Jenkins (4) was classified as of this form genus in 1925 (4). It is thought that this data, together with the fact that the fungus concerned is a Sphaceloma (4), may serve further to explain or elucidate unsolved matters relating to the dissemination and distribution of this organism (11, p. 12). It is, of course, already recognized that this pathogene may be borne by wind (3) and by rain (11) or by these two natural agencies in combination (11). Also, there is available no little information concerning the environmental conditions under which the fungus may persist (6, 7, 11). Inasmuch as in Florida, at least, avocado, *Persea* spp., and Citrus are grown in the same nursery, or grove situations, the Sphaceloma (10) causing avocado-scab (5) would naturally occur in close proximity to the structurally similar species affecting Citrus. It would therefore seem probable that, to a greater or less extent, the data here presented might also apply to the Sphaceloma pathogenic on avocado, observed under corresponding conditions.

[1] The work reported in this paper was done in cooperation with the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and with the Department of Plant Pathology, Florida Agricultural Experiment Station.

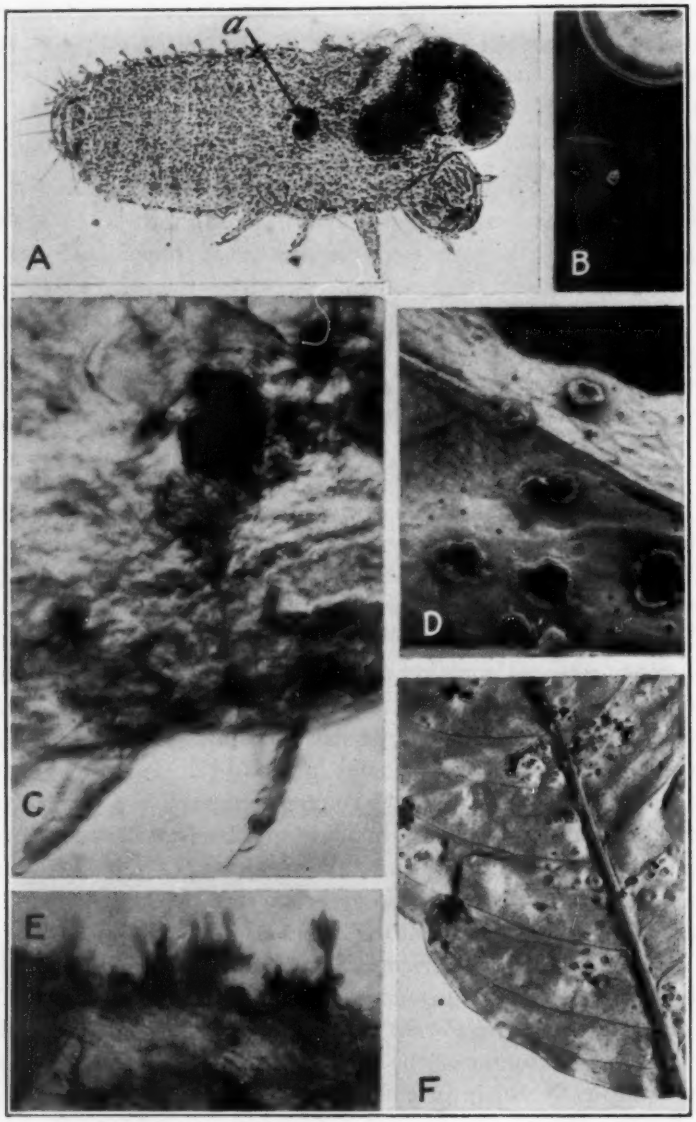

FIG. 1. A, insect discovered feeding upon conidia and conidiophores of the Sphaceloma (X approx. 85). B, part of a colony of *Cladosporium* sp. and an entire colony of the Sphaceloma, in original isolation culture (X 5/6); C, thoracic region of insect showing fructifications of the Sphaceloma scattered about the frontal end of the stomach (X approx. 300); D, enlargement of a group of scab lesions on lower part of F (X 6); E, vertical section through lesion (necrotic) showing the Sphaceloma characteristically fruiting on the lower leaf surface and, in the clear spaces in the leaf structure, intertwined hyphae of this fungus (X approx. 160). F, general appearance of Sphaceloma-covered lesions on lower surface of fresh leaf (X 5/6). B and E illustrations by J. F. Brewer; the others, by Marcel L. F. Foubert.

While examining fresh young leaves of the sour orange, *Citrus aurantium* L., quite by chance a small insect was observed in the act of feeding upon the luxuriant growth of the Sphaceloma covering the lesions or galls characteristically produced on the lower leaf surface. The leaf was one selected at random from a large assortment of about two-weeks-old leaves and stems gathered from sour-orange trees in the rutaceous collection at the Florida Agricultural Experiment Station at Gainesville. A sample of leaves from this gathering, similar to the one on which the insect was found, is shown in figure 1, D–F.

The examination, referred to above, was being made in connection with an investigation of the natural development of the Sphaceloma on fresh young growth of Citrus. This study was based not only on the rutaceous collection at Gainesville, but also on the one at the United States Department of Agriculture Citrus Diseases Field Laboratory at Orlando. The work was begun early in September and for the first three weeks was done in the field; subsequently, that is, until the end of November, it was done at Washington, D. C., where, through the kindness of Professors G. E. Weber and H. E. Stevens, and Mr. J. F. Wootten, fresh specimens of various Citrus varieties were received at desired intervals.

Only about 0.7 mm. in length, light colored and somewhat transparent in the stage observed, the insect might easily have been overlooked but for the fact that the lesions were being examined either with a hand lens or binocular microscope. From the position it assumed on the galls and the movements of its head, there seemed no question that it was feeding on the mass of dark, fuscus-color[2] conidia and conidiophores of the Sphaceloma. After busily pasturing on a given lesion for a short time, although an abundance of food still remained, it would turn quickly about, hasten to another lesion, climb up the raised margin and upon reaching the dark fungus-covered area, immediately engage in its feeding as before. This procedure had evidently been in progress for some time when the insect was first observed, for even then, because of the transparency of its rather flexible body, the stomach was visible as a nearly black, solid-appearing structure which, later examination showed, was completely filled with conidial fruiting structures of the Sphaceloma similar to those illustrated in figure 1, E.

A few moments after its feeding was thus observed, the insect was removed from the leaf, killed in alcohol, and mounted in glycerine jelly on a glass slide. Microscopic examination of the contents of the stomach was facilitated because during the process of killing and mounting, the organ became so ruptured and displaced that most of it protruded from the soft

[2] Color reading based on Ridgway (9), made by J. Marion Shull.

flexible body of the insect at a point just above the head (Fig. 1, A). Only the frontal end remained in place (Fig. 1, A, *a*; also see Fig. 1, C). The stomach was now seen to be closely packed with many hundreds of characteristically colored conidia and conidiophores of the Sphaceloma such as those illustrated in figure 1, E. It could not be determined that alimentation to this point had in any way changed the appearance of the fungus. In fact, where the conidiophores were thinly scattered, as they were about the frontal end of the stomach incident to the rupture of the canal, it was observed that they were still almost intact, some of them with the conidia apparently still attached (Fig. 1, C). Voided pellets containing this growth, and at the same time definitely known to be those of this insect, were not available, therefore, the viability of the Sphaceloma after alimentation by the insect could not be determined.

Mounted as described, the insect was identified by Dr. Adam Böving, Bureau of Entomology, United States Department of Agriculture, as the larval stage of one of the Mycetaeids, a little-known group of Coleopterous insects. The mounted specimen has been deposited in the insect collection at the U. S. National Museum.

All of the other sour-orange leaves and stems constituting the specimen on which the insect was found, as well as the specimens of sour-orange and other Citrus varieties subsequently received, were carefully inspected, either by the writer or by Dr. Böving, but no more Mycetaeids or indications of their having been present were seen, although other small insects, such as Coccids and gall mites, or their remains, were observed. These insects or remains of insects were sometimes found in close association with the lesions. A small fly also was present, moving about on most of the specimens when the packages containing them were opened. Also, while in Florida, the writer noted that medium-size ants were often traveling about over those Citrus plants under examination. No attempt has been made to determine whether any of these insects are concerned in the transportation of the fungus. Available information dealing with ants and flies (1), or with ants alone (8), as carriers of other organisms pathogenic on plants, suggests, however, that these insects, and perhaps others as well as the Mycetaeid, may be concerned in the dissemination of the Citrus Sphaceloma.

It is, of course, possible that various insects are attracted to this fungus or to related fungi as food on account of the mucilaginous character of the walls and the presence of an occasional free, glistening, viscid substance[3] derived from them in which conidia may be embedded.

[3] Jenkins, A. E., (Glistening substance covering growth of *Sphaceloma fawcettii* Jenkins, *Sphaceloma ampelinum* de By., and *Plectodiscella veneta* (Sacc.) Burk.) In Fawcett, H. S., and H. A. Lee, Citrus diseases and their control. p. 490. 1926.

At any rate, observations made in connection with numerous examinations of scab lesions on young Citrus suffice to indicate that insects of one kind or another feed more or less commonly on the Sphaceloma normally present on such lesions. For instance, here and there, fully developed lesions were noticed from which some or all of the exposed growth of the fungus and even the underlying stromatic layer, as well as dead host tissue intermixed with hyphae, situated immediately beneath this layer, had prematurely disappeared as though cropped or gnawed by insects. On or about these lesions were insect feces containing free Sphaceloma conidia, as well as conidiophores and fragments of stroma presumed to be also of this

FIG. 2. Confluent lesions on leaf showing patch from which the fungus growth has evidently been eaten by insects (X 6). Photograph by J. F. Brewer.

fungus. In some cases, entire conidiophore tufts like those shown in figure 1, E, were seen, some of them still attached to the stromatic base on which they were borne. In addition, conidia and hyphae or conidiophores thought to be those of *Cladosporium* sp. were present in the feces examined, such hyphae or conidiophores often protruding beyond the rounded surfaces of these feces. The confluent lesion illustrated in figure 2 shows the appearance of Citrus-scab lesions on which insects have evidently fed. On the lower part of the patch labeled *a*, represented as small black dots, are feces of insects as present on this particular leaf of the sour orange received from Orlando late in November.

Isolations were made from two individual pellets taken from the patch illustrated in figure 2, *a*, and from each pellet the Sphaceloma was obtained in pure culture. The method of isolation was as follows: Under as nearly aseptic conditions as possible, each pellet was several times washed in an abundance of water, then macerated in a drop of water on a glass slide and examined with the compound microscope to make certain that Sphaceloma conidia were present. From this maceration, dilution plate cultures on potato-dextrose agar were made. In due time from the two sets of cultures, one from each pellet, there developed characteristic colonies of the slow growing Sphaceloma, as well as of the faster growing *Cladosporium* sp. There was little admixture of other fungi or bacteria (Fig. 1, B). The Sphaceloma isolated in this manner from feces of insects was culturally so similar to other strains of *S. fawcettii* isolated direct from lesions as to leave no question of its identity with this species.

Thus, conidial fructifications of *Sphaceloma fawcettii* on a fresh young leaf of Citrus were fed upon by a small larva classified as one of the Mycetaeids, a little-known group of beetles. Lesions of Citrus scab also were found from which some or all of the Sphaceloma growth had been eaten away by insects of one kind or another. And, from feces of insects of unknown identity present on such lesions, the Sphaceloma and *Cladosporium* sp. were isolated and grown in artificial pure culture. It must be admitted that the data here presented have not definitely proved that the particular Mycetaeid and perhaps some of the other insects here specifically named are carriers of the Citrus Sphaceloma. The assertion can be made, however, that some insect feeding on *Sphaceloma fawcettii*, the cause of Citrus scab, is evidently capable of acting as a transporting agent for this pathogenic fungus by passing it in a viable condition through the intestinal tract.

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A PROBABLE EXPLANATION OF RECENT EPIDEMICS OF BUNT IN DURUM WHEATS[1]

C. S. HOLTON[2]

Extensive studies on varietal resistance of wheats to *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn have indicated that there are wide differences in susceptibility. Gaines (1) and Stakman, Lambert, and Flor (6) called attention to the fact that, while there are great differences in the relative susceptibility of varieties within the common wheat group and some differences within the durum group, the durums as a class appeared to be more resistant than the common-wheat group in the United States.

For many years the durums seemed to be far more resistant than the common wheats in the hard red spring-wheat region of the United States, although Marquis, the most commonly grown hard red spring wheat, is highly resistant. Beginning about 1925, however, numerous complaints were made that the durums were smutting rather generally. As a matter of actual fact, bunt in durum became rather acute. It seemed to be causing greater damage in durums than in common wheats. After the outbreak of bunt in 1925, Rodenhiser and Stakman (5) suggested that it might have been due to unusually favorable soil and weather conditions for the development of bunt or to the presence of a form of the pathogene that was unusually virulent on the durums. Rodenhiser and Stakman (4, 5), Gaines (1), and Reed (2) had shown that there are physiologic forms, both of *T. tritici* and *T. levis,* which could be recognized by their virulence on certain varieties of wheat, although they found none that was especially virulent on durums. Reichert (3) called attention to the fact that in Palestine the durums actually seemed to be more susceptible to the form of bunt present there than the common wheats, which would indicate the existence of forms that attack durums severely. It seemed quite likely, in view of the increasing severity of bunt on durum varieties in the United States and in view of Reichert's observations in Palestine, that the true explanation of the outbreak of bunt in the spring-wheat region of the United States might be due to the presence of unusually virulent strains of the pathogene. Therefore experiments were made to determine whether this was true.

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[2] The writer is indebted to Dr. E. C. Stakman for suggestions and criticisms and to Dr. J. J. Christensen and Dr. H. A. Rodenhiser for suggestions offered during the course of the investigations.

It is doubtful whether the term physiologic form can be applied properly to chlamydospore collections of bunt. There seems to be evidence that many of the smuts are heterothallic; therefore, chlamydospores would represent the diploid phase, which cannot be propagated independently. When the spores germinate there presumably are reduction division and segregation of sporidia into two or more sexual groups. Hybridization would again occur before chlamydospores could be produced on the plants. If this be true, perhaps only monosporidial lines would be designated properly as physiologic forms, and the chlamydospores would be hybrids between forms. On the other hand, Tilletia may possibly be homothallic or, if heterothallic, monosporidial lines may be similar except in sex. If this be true, the objection would not hold. For practical purposes it seems justifiable to consider as physiologic forms such collections of bunt as were used in this paper; but, from the scientific viewpoint, there is some question as to whether they may properly be so designated. In any case there are decided differences in the virulence of different chlamydospore collections, and this fact has been demonstrated to have considerable significance both in the epidemiology of bunt and in the development of resistant varieties. It may be useful, therefore, to designate the different collections showing different degrees of virulence as physiologic forms, and they are so designated in this paper.

MATERIALS AND METHODS

Eight varieties of common and durum wheats and of emmer were inoculated with the spores of five collections of *Tilletia tritici*, as shown in table 1. The seed was treated with formaldehyde, washed in water, and, when dry, inoculated at the rate of 0.5 gram of spores to 100 grams of seed. After inoculation the seed was sown in triplicate, systematically distributed, eight-foot rows in the field.

The percentages of infection (Table 1) are based on counts of 900 heads in all varieties except Mindum. In this case the percentages are based on the total number of heads, ranging from 613 to 704, in three eight-foot rows. Except for Kota, Preston, and Marquis, in which smutted heads were easily detected by external examination, smutted heads were determined by clipping each head three times. All heads containing smut were recorded as smutted regardless of whether they were partially or completely so.

RESULTS

The results are summarized in table 1. The Washington, California, and Manitoba collections did not show sufficient differences in virulence on the eight varieties used to warrant separating them into distinct physiologic forms. For the present they are considered identical, although they may

TABLE 1.—*The percentage of smutted heads in eight varieties of wheat and emmer inoculated artificially with five collections of Tilletia tritici at University Farm, St. Paul, Minnesota, 1929*

Class, variety, and C. I. number	Source of inoculum and percentage of smutted heads				
	Washington	California	Manitoba	Devils Lake, N. Dakota	Litchfield, Minnesota
Common					
Kota–C. I. 5878	42.2	53.8	42.6	43.3	14.7
Preston–Minn. 924	7.4	11.6	16.1	2.3	0.9
Marquis–C. I. 3641	1.5	3.3	4.9	0.1	0.1
Marquillo–C. I. 6887	1.2	1.4	2.7	0.1	0.0
Hope–C. I. 8178	0.0	0.0	0.0	0.0	0.0
Durum					
Mindum–C. I. 5296	2.6	4.6	3.5	50.6	3.3
Pentad–C. I. 3822	12.2	8.4	8.9	41.8	9.9
Emmer					
Vernal–Minn. 1524	1.2	1.3	0.0	8.1	23.6

possibly differ in virulence on other varieties. The collection from Devils Lake, North Dakota, was extremely virulent on Mindum and Pentad (50.6 per cent and 41.8 per cent, respectively), while the remaining varieties, except Kota, were highly resistant to it. As the table indicates, the collection from Litchfield, Minnesota, shows a comparatively low degree of virulence on Kota (14.7 per cent) and a fairly high degree of virulence on Vernal (23.6 per cent). All other varieties used, except Pentad, were highly resistant to this collection. Hope was immune from all five collections.

It is obvious from the results graphically shown in figure 1 that there are three distinct physiologic forms of *T. tritici* in the five collections used. The Washington, California, and Manitoba collections were not sufficiently different in virulence to justify separating them into distinct forms and they are, therefore, considered as the same form. Rodenhiser (4) suggested that the above-named collections might be diffrent physiologic forms, but his results were not convincing enough to warrant the establishment of different forms. The fact that the Washington, California, and Manitoba collections are here considered as a distinct physiologic form does not mean that they represent a form different from form 1 or form 2, described by Rodenhiser (4), but rather that they represent a form distinct from the other two herein reported.

Undoubtedly, the collection from Devils Lake, North Dakota, is a distinct physiologic form that can be distinguished readily by its reaction on Min-

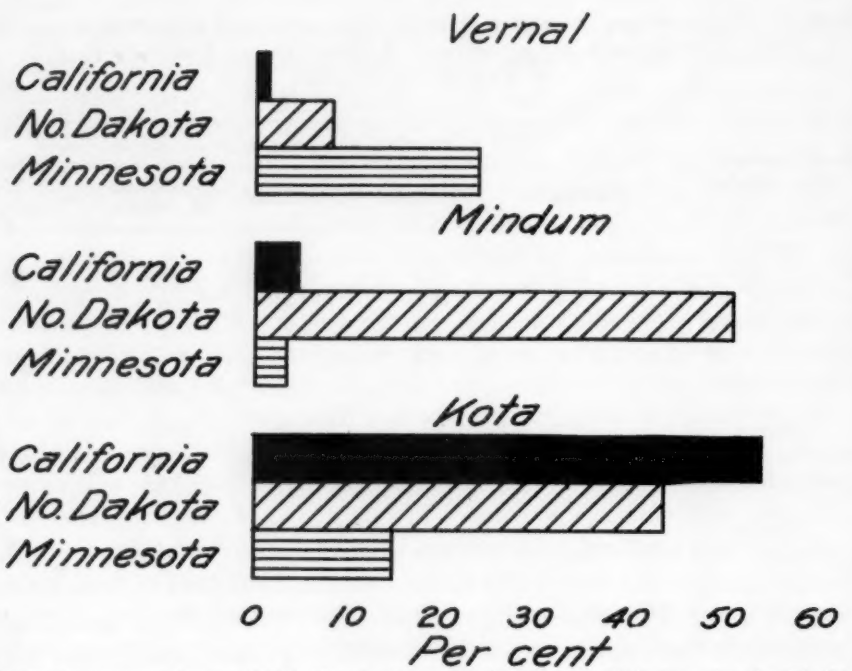

FIG. 1. The percentages of infection in Vernal, Mindum, and Kota inoculated with the spores of three collections of *Tilletia tritici* in 1929.

dum and Pentad. This form apparently was either not present or prevalent prior to about 1925. Whether it was introduced from some other region or resulted from hybridization or mutation cannot, of course, be stated.

The Litchfield, Minnesota, collection obviously represents still another physiologic form, readily distinguishable by its reaction on Kota and Vernal. Kota, extremely susceptible to the other four collections, is moderately resistant to the Litchfield form; while Vernal (emmer), resistant to all other collections of smut used in these tests, is quite susceptible to the Litchfield form. Consequently, Vernal can no longer be considered resistant to bunt.

The phenomenon of physiologic specialization in *T. tritici* is important from a practical as well as a purely scientific standpoint. One can never be certain that resistant varieties of wheat, developed for certain localities, will remain resistant indefinitely, since new physiologic forms may appear at any time and render hitherto resistant varieties completely susceptible.

SUMMARY

1. The virulence of five collections of *T. tritici* was compared on eight varieties of Triticum: common wheat, durum, and emmer.

2. Three distinct physiologic forms were distinguished on the basis of pathogenicity. The Washington, California, and Manitoba collections represent one form which is distinguished by its high virulence on Kota and low virulence on the other seven varieties. The collection from Devils Lake, North Dakota, represents a form characterized by its marked virulence for durum wheats, and the Litchfield, Minnesota, collection represents a third form, distinguished by its high virulence on Vernal.

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THE RELATIVE TRANSPIRATION RATE AT INFECTION SPOTS ON LEAVES

R. B. Harvey

It has been reported by the writer[1] that the accumulation of nontoxic dyes can be used to indicate differences in the rate of transpiration from leaf surfaces. The feasibility of this method was checked by methods which, normally, should produce a high rate of transpiration in certain spots on the leaf, such as by blowing onto it a current of air from a fine nozzle and by focusing the light from a Mazda lamp on the leaf by means of a lens.

In spots over the leaf blade where, by other methods, it seems demonstrated that the transpiration rate is higher than elsewhere, there is an accumulation of Light Green S.F. and other nontoxic dyes which penetrate easily into the vascular system of the leaf. Using this dye-accumulation method it was attempted to demonstrate local differences in transpiration rate over the leaf blade and on stems in certain cases of spot infections by fungi.

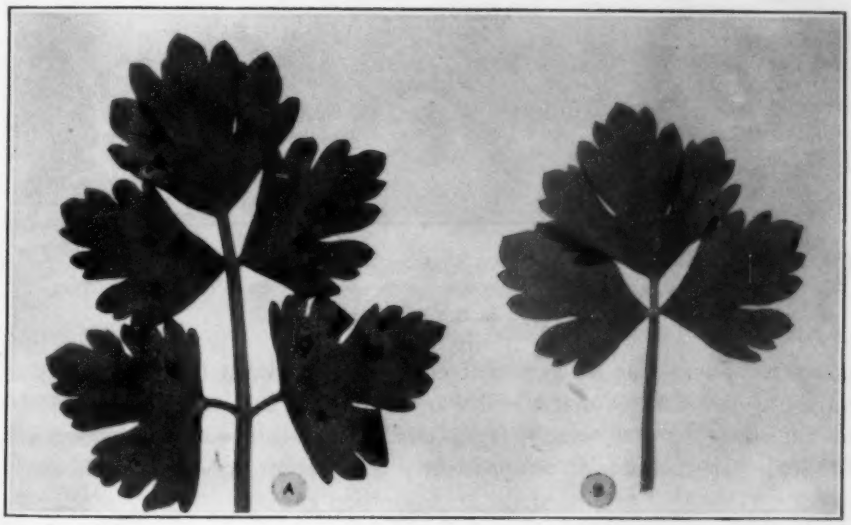

Fig. 1, A. A leaf of celery with Septoria infection spots and dye accumulation around them. Photograph through color screen to show dye in black. B. A normal celery leaf showing uniform distribution of dye. The black areas are caused by double thickness of the leaf.

[1] Proceedings of the American Society of Plant Physiologists, December, 1929.

Local infections by Septoria on celery leaves (Fig. 1, A) show dry, brown, corky areas of cells, killed by the fungus. When the petioles of infected leaves are placed in 0.1 per cent aqueous solution of Light Green S.F., or certain other nontoxic dyes, there is an accumulation around the infection spots. The brown, corky spots do not accumulate dye in a zone so much as a millimeter or so wide in the surrounding live cells. Evidently, the dead cells do not evaporate the aqueous dye solution so rapidly as do the areas into which the fungus is spreading. A leaf with no infection spots (Fig. 1, B) shows a uniform coloration of the dye.

Leaves of Hubbard squash, infected with spots of mildew (*Erysiphe* sp.) (Fig. 2), on both upper and lower surfaces, were tested in a similar

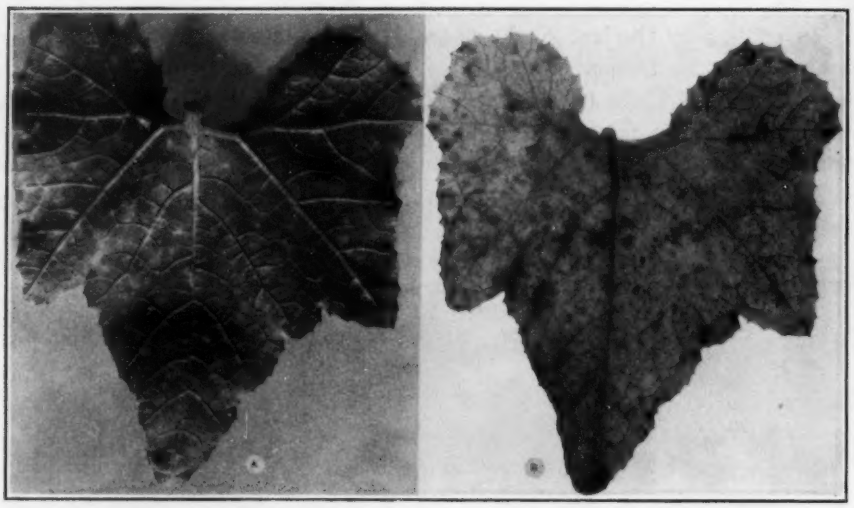

FIG. 2, A. A leaf of Hubbard squash showing areas of mildew on the lower surface. B. The same leaf photographer through a screen to show the accumulation of dye in the mildewed areas.

manner. There was no apparent correlation between the infection spots on the upper surface and the dye accumulation, but the spots of mildew on the lower surface were sharply outlined by deep-colored areas within the leaf. In this case it was evidently the presence of the fungus on the lower surface that increased the transpiration rate.

In the case of rust pustules of *Puccinia graminis* on wheat (Fig. 3, A), there is an accumulation of dye around the pustules. The color is especially deep in these areas where the epidermis is broken.

In squash leaves punctured by Aphis, the accumulation of dye was sharply limited to the affected area (Fig. 3, B), indicating that a high

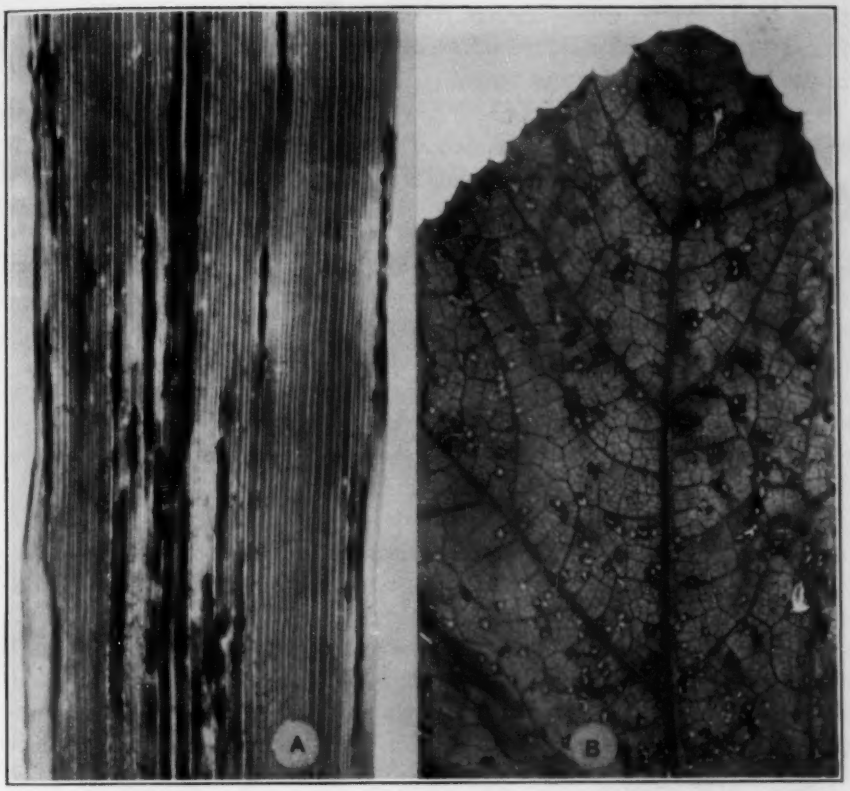

FIG. 3, A. Accumulation of dye around pustules of *Puccinia graminis* on wheat. B. A squash leaf showing insect punctures outlined by areas of dye accumulation.

transpiration rate occurred in those areas. When the leaf was ashed in the muffle furnace, there was left a perfect skeleton of the leaf structure, showing the cell walls. In the areas in which dye accumulation had occurred, there was much more abundant residue of a light grey ash than elsewhere over the leaf. This was found to be the case whether the leaves were placed in dye solution or not. Evidently the rapid transpiration at the injured spot is associated with an accumulation of ash constituents in the tissue.

SUMMARY

It is considered that the accumulation of such nontoxic dyes as Light Green S.F. can be used in demonstrating the effect of certain local infec-

tions by fungi on transpiration from the leaf. Insect punctures were found to cause local accumulations of dyes and also of ash constituents in the immediately adjacent tissue.

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PHYTOPATHOLOGICAL NOTES

The control of carnation rust, Uromyces caryophillinus (Schr.) Wint., with sulphur.—In the light of results obtained by several investigators, Kolodust, a product of the Niagara Sprayer Company, was accepted as a suitable form of sulphur. The carnation varieties used in the dusting experiments were Arctic and Radiolite. The work was carried out in a greenhouse, maintained approximately under commercial conditions. The plants were grown on standard greenhouse benches. Fertilizer was applied in the dry state and the water supplied so that the foliage never became wet or spattered. The plots were separated by double thicknesses of fine cotton cloth supported by rigid wooden frames. The dust was applied with a hand duster in quantity measured only in terms of a uniform coverage per application. The applications of sulphur were made at intervals of one, two, or three weeks. In addition, all infected leaves were hand-picked in one plot that was dusted weekly. The experimental period extended from November 27 to April 15. Thermograph records were kept from December 4 to the close of the period. In general, there were relatively few days when the temperature did not reach 70° F. for at least two hours of the 24-hour interval. In order to obtain a measure of rust infection and its spread, all plants were examined for leaf infections at the beginning and close of the experiment. The records were kept in terms of infected leaves per plot. Yield was recorded in numbers of cut flowers per plot.

The results indicate that carnation rust can be controlled by the use of Kolodust. An interval of two weeks per application is apparently as effective as one week. This accords with inoculation experiments, in which nine varieties were artificially inoculated. Rust pustules appeared in from 17 to 19 days. Data based on a limited number of controlled inoculations in nine varieties indicate a wide range in the rust resistance of carnation varieties. Dusting at three-week intervals gave no control. The weekly application, accompanied by the removal of infected leaves, proved inadequate. It is thought that hand-picking may have increased the number of infections through spore dissemination. Accepting the flower yield of the control plot as 100 per cent, the plot receiving dust at two-week intervals yielded 258 per cent. Some care was exercised to avoid dusting the petals of partially opened buds. In no case was there any noticeable direct injury to the flowers. Red spiders, *Tetranychus telarius* L. were not found on sulphur-dusted plants though they were abundant elsewhere in the greenhouse. This confirms the experience of entomologists. Near the end of the experimental period the treated plants showed signs of injury and

many individuals had died by May 15. The source of injury was found to be at the roots. Hydrogen-ion determination gave pH readings of 5.5 for the nontreated soil and 2.4, 2.9, and 3.2 for the soil receiving Kolodust at one-, two-, and three-week intervals, respectively. The rate of mortality per plot was in accord with the frequency of dust application. Since it is known that carnations are favored by applications of lime, it is believed that proper applications of agricultural lime would correct the acidity and thus protect the plants.—F. H. STEINMETZ, College of Agriculture, Orono, Maine.

The occurrence of the ring-spot disease of sugar cane in Florida.—On August 28, 1929, while the writer was making a disease survey of a small sugar-cane plot near Indiantown, Fla., typical cases of the ring-spot disease were observed on leaves of the variety D. 109. A microscopic examination of numerous perithecia, asci, and spores showed agreement in spore dimensions with published descriptions of the fungus *Leptosphaeria sacchari* van Breda de Haan. Comparison also was made with authentic herbarium material of this disease collected in April, 1923, by the writer, in Porto Rico. By these means, the identity of the disease was established beyond doubt.

Concerning the distribution of ring spot, Bell[1] has recently summarized the available information. He shows the disease present in Hawaii, Fiji, Australia, Philippines, Java, India, Mauritius, South Africa, Cuba, Porto Rico, British West Indies, Brazil, Argentina, and Peru.

So far, the disease has been found only near Indiantown, at Canal Point, and Belle Glade, Florida. The varieties D. 109, D. 117, Co. 281, C. P. H. 139, Crystalina, and several seedlings, bred at the U. S. Experiment Station at Canal Point, have been found heavily infected, although only the lower leaves were involved and the loss at present is regarded as negligible. The disease has not been found in the large commercial sugar-cane plantings at South Bay, Clewiston, Liberty Point, and Benbow, around the south end of Lake Okeechobee. Apparently, only the regions most severely swept by the central vortex of the hurricane of September, 1928, are infested. This would seem to support the theory that living spores were carried by the hurricane from Porto Rico, where the disease is widely distributed.

Prevalence of the disease on the lower leaves of some of the noncommercial seedlings at the Florida Agricultural Experiment Station at Belle Glade makes it evident that future attention will have to be directed toward

[1] Bell, Arthur F. A key for the field identification of sugar cane diseases. Queensland Bur. Sugar Exp. Stas., Div. Path. Bul. 2. 1929.

breeding for resistance to this disease. Abbott[2] has recently recorded that two cases of severe leaf injury were reported in Peru in 1927. The plants were said to be nearly mature, however, so that only slight loss took place. Bell[1], on the other hand, has already pointed out the potential importance of the disease and cites as an example the present great economic importance of eye spot both in Hawaii and Porto Rico, due to the commercial use of certain very susceptible varieties.—B. A. BOURNE, *Pathologist, Research Department, The Southern Sugar Company, Clewiston, Florida.*

[2] Abbott, E. V. Diseases of economic plants in Peru. Phytopath. **19**: 645–656. 1929.